CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4



H. K. LEWIS AND CO., LTD., 136, GOWER STREET, LONDON, W.C. I
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
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THE JOURNAL OF AGRICULTURAL SCIENCE

EDITED FOR THE PLANT BREEDING AND ANIMAL NUTRITION RESEARCH INSTITUTES AT CAMBRIDGE, AND THE ROTHAMSTED RESEARCH INSTITUTES BY

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VOLUME XX 1930

CAMBRIDGE
AT THE UNIVERSITY PRESS
1930

PRINTED IN GREAT BRITAIN

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VOLUME XX JANUARY, 1930 PART 1

A STUDY OF NITROGEN METABOLISM IN SHEEP ON HIGH PROTEIN DIETS.

BY JAMES STEWART, M.A., B.Sc., PH.D.
(*Department of Animal Pathology, Cambridge University.*)

(With Five Text-figures.)

IN the course of an investigation into the pathological effects of excessive protein diets on sheep (1) experiments were designed to show the changes in metabolism as recorded by nitrogen balances and weight curves. The animals were placed in metabolism cages similar to those described by Wood and Woodman (2) in their digestibility trials, and a harness of the Halnan type used.

Before describing the actual method of conducting the experiments, the method employed for sampling the feed composing the rations should be mentioned. The foodstuffs were all ordered in large amounts, such as 4 or 5 cwt. The whole amount was mixed and sampled for analysis by quartering. This method was considered to be sufficiently accurate since it was found by repeated experiments that the composition of the material varied very slightly in bulk. The foodstuffs were all kept in a cement fodder-house and it was found that even the proportion of the dry matter varied only to a small degree. Sufficient mangolds were selected for one experiment and were sampled for dry matter and chemical analysis by boring. This manner of dealing with the foodstuffs was maintained throughout the experiments and was repeatedly checked so that no error could arise in the estimation of the consumption and excretion of nitrogen. The food of the ration was weighed out each morning from bulk and in the case of grass, the composition of which was varying daily, the methods described by Woodman, Blunt and Stewart (3) in their pasture digestibility work were employed. The dry matter was estimated daily and a composite bi-weekly sample of the grass was analysed.

Each morning the amount of faeces was weighed and the volume of urine measured. An amount corresponding to one-fifth of the daily faeces and one-tenth of the daily urine was kept in cold storage and a bi-weekly composite sample made up which was then analysed. The food, as stated, was weighed out every morning and the residue which

was gathered twice a week was measured and analysed. In this way an accurate bi-weekly nitrogen balance was maintained so that a careful record of the protein metabolism (as far as balance work can show) was made.

Composition of the foodstuffs used in the experiments.

As has been previously stated the foodstuffs were ordered in sufficient quantity to last 2 or 3 months and were sampled from bulk for analysis. To check this method of sampling, several analyses were made of each bulk of foodstuffs throughout the period it was used.

Table I shows a comparison between different analysis of one bulk with analysis of different bulks of linseed cake.

Table I. *Dry matter* = 100 *per cent.*

	A			B		C	
Crude protein	26·80	26·86	26·84	29·61	29·64	31·00	31·01
Ether extract	9·93	9·91	9·92	8·73	8·74	9·54	9·51
Fibre	8·70	8·72	8·11	9·31	9·31	8·96	8·95
Ash	6·25	6·01	6·10	8·54	8·41	7·22	7·21
Nitrogen-free extractives	48·31	48·49	48·41	43·79	43·87	43·27	43·29
Organic matter	93·75	93·99	93·90	91·46	91·59	92·78	92·79
Dry matter as analysed	88·00	88·04	88·16	87·42	87·31	90·06	89·21

The above table shows that the variation in one bulk was very small whilst between two bulks the composition of the cake varied quite considerably. The slight variation in the three analyses of one bulk at different periods in A, and the corroboration given in the cases of B and C, the writer thinks is ample evidence to show that the method of sampling mentioned is sufficiently accurate to warrant its use in metabolism work. It relieves the investigator of the large amount of work which a daily or bi-weekly analysis of the ration entails.

Details of the nitrogen balances and change of weight of sheep on high protein diets.

In case of Sheep I and Sheep II the change of weight during experiment was not recorded. The diet of Sheep I and Sheep II consisted of mangolds, hay, barley meal and decorticated cottonseed cake meal. The barley meal was gradually eliminated and the cottonseed cake increased proportionately, thus obtaining an increasing protein ration. Table II gives the results for the mean daily nitrogen consumption, nitrogen excretion, and nitrogen balances of Sheep I in the several composite periods. For purposes of discussion the tables are shown in graphical form in Fig. 1.

Table II. *Showing mean daily nitrogen balance of Sheep I.*

Composite	Nitrogen consumed per day gm.	Nitrogen voided per day: Faeces gm.	Urine gm.	Total gm.	Mean daily nitrogen balance gm.
1	22·13	6·90	10·52	17·42	+4·71
2	16·97	4·84	9·57	14·41	+2·56
3	10·25	2·87	9·51	12·38	− 2·13
4	11·67	2·54	6·46	9·00	+2·67
5	13·87	2·65	5·41	8·06	+5·81
6	16·02	2·73	6·78	11·51	+4·51
7	20·48	4·59	8·16	12·75	+7·73
8	21·69	5·56	8·32	13·88	+7·81
9	23·84	5·22	10·62	15·84	+7·80
10	24·74	5·73	11·88	17·61	+7·13
11	25·38	5·83	11·56	17·39	+7·99
12	27·05	6·59	14·11	20·70	+6·35
13	27·94	7·43	12·40	19·83	+8·11
14	27·03	6·25	13·61	19·86	+7·17
15	28·31	7·22	14·68	21·90	+6·41
16	29·57	7·33	14·34	21·67	+7·90
17	29·85	7·87	16·59	24·46	+5·39

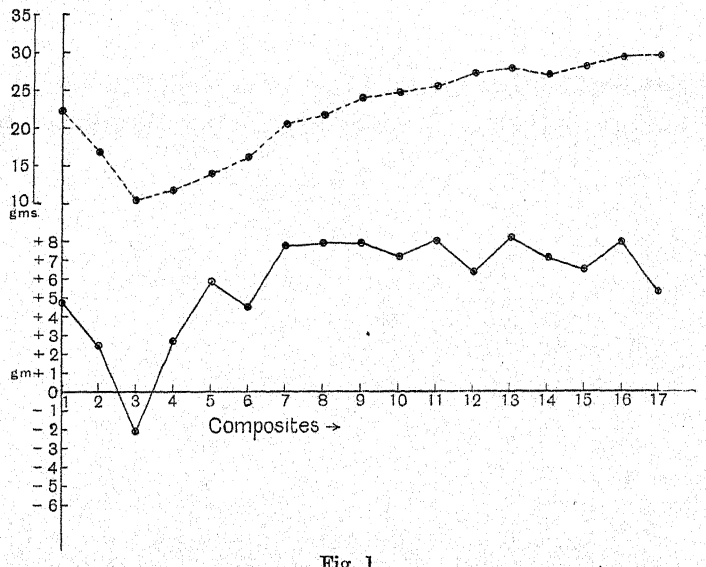


Fig. 1.

..... Nitrogen consumption ——— Nitrogen balance

As seen from Fig. 1, Sheep I shows an initial negative balance. This was found to be general for all the sheep on experiment, and is due, no doubt, to the effects of cage conditions on the animals. The fall to a negative balance runs parallel to a fall in consumption of the ration. During the rest of the experiment Sheep I had an increasing nitrogen

intake, increasing from a daily intake of 10·25 gm. to 29·85 gm. nitrogen. The balance during this period increases from − 2·13 gm. to + 7·80 gm. in 14 days. At this figure it remains practically constant, with the exception of a few slight variations, during the remaining 5 weeks of the experiment. During the 5 weeks its daily consumption increased from 20·47 gm. nitrogen to 29·88 gm. nitrogen which corresponds to an increase in protein (nitrogen value × 6·25) of 59·22 gm., the greater portion of which is derived from the cottonseed cake fraction of the ration.

It seems from these figures that above 20·47 gm. nitrogen intake, the animal made no use of the extra protein ingested, at least as far as regards retaining it for body-building purposes. This agrees with the findings of Crowther and Woodman (4) who found that, in the case of sheep, there seemed to be an optimum nitrogen intake point coinciding with a maximum retention point, which differed for different animals. These writers, however, found that once the maximum retention point was reached the animals showed a tendency to assume a state of nitrogenous equilibrium, and not, as in the case of Sheep I, show a constant retention.

Crowther and Woodman used quite mature animals for their experiments, while Sheep I was quite immature and would thus be expected to show a protein retention. While Sheep I showed the remarkably constant daily retention of nitrogen, a clinical examination of its urine showed a periodic appearance of albumin, which did not coincide with any periodic variation in protein consumption. The constancy of the nitrogen retention figure during this periodic albuminuria is of decided interest.

Sheep II proved disappointing as an experimental animal and its nitrogen metabolism as recorded by balance tables and curves is of little interest.

Table III. *Showing mean daily nitrogen balance of Sheep II.*

Composite	Nitrogen consumed per day gm.	Nitrogen voided per day			Mean daily nitrogen balance gm.
		Faeces gm.	Urine gm.	Total gm.	
1	22·40	7·98	7·92	16·90	+5·50
2	22·38	7·68	9·41	17·09	+5·29
3	21·93	7·21	10·56	17·77	+4·16
4	22·82	7·90	9·65	17·55	+5·27
5	18·00	5·10	7·83	12·93	+5·07
6	16·80	3·80	8·23	12·03	+4·77
7	16·46	3·13	7·12	10·25	+6·21
8	16·33	3·96	8·78	12·72	+3·61
9	15·26	3·49	9·08	12·57	+2·69
10	17·11	3·52	8·15	11·67	+5·44

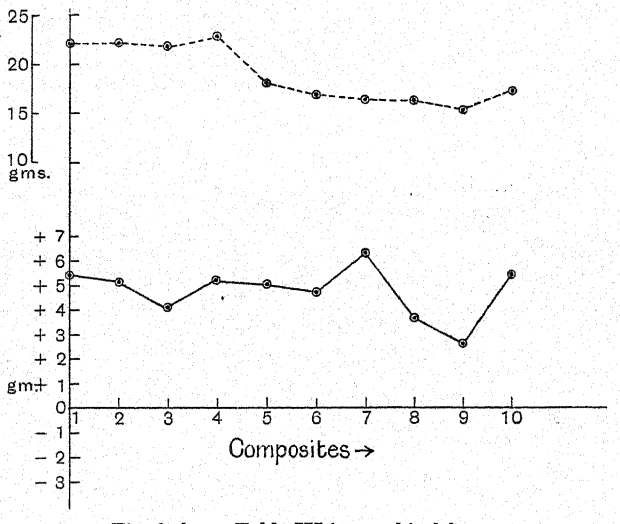


Fig. 2 shows Table III in graphical form.

- - - - - - - - Nitrogen consumption ———— Nitrogen balance

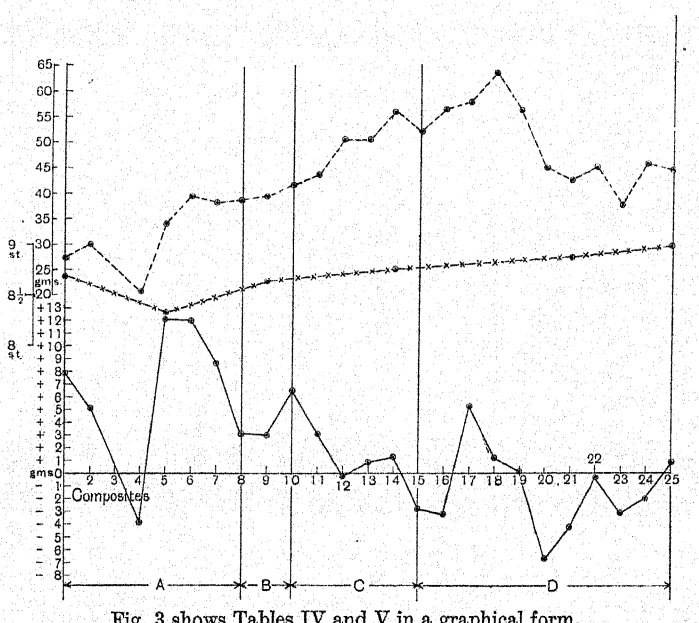


Fig. 3 shows Tables IV and V in a graphical form.

- - - - - - - Nitrogen consumption ×-×-× Change of weight

———— Nitrogen balance

Sheep II very soon became accustomed to cage conditions and ate its food greedily, and on this account proved all the more disappointing when its appetite fell off and its consumption decreased rapidly. The sole point of interest is the fact that during a heavy haemoglobinuria and albuminuria in composites 8 and 9, the nitrogen balance showed a tendency to fall, but in composite 10, where the appearance of albumin in the urine was heaviest, the nitrogen balance increased. This considered in the light of the balance of Sheep I points to no apparent correlation between the periodic albuminuria and the retention of nitrogen by the animal.

Table IV. *Showing mean daily nitrogen balance of Sheep III.*

Composite	Nitrogen consumed per day gm.	Nitrogen voided per day			Mean daily nitrogen balance gm.
		Faeces gm.	Urine gm.	Total gm.	
1	27·43	6·40	13·04	19·44	+7·99
2	30·00	10·40	14·45	23·85	+5·15
3	—		Animal scouring		—
4	20·85	9·35	15·35	24·70	− 3·85
5	34·35	8·98	13·24	22·22	+12·13
6	39·15	8·95	18·16	27·11	+12·04
7	38·04	11·31	17·96	29·27	+8·77
8	38·52	9·38	26·11	35·49	+3·03
9	39·26	9·52	26·70	36·22	+3·04
10	41·56	8·74	26·33	35·07	+6·49
11	43·75	9·58	30·99	40·57	+3·18
12	50·32	11·62	38·85	50·47	− 0·15
13	50·29	10·55	38·88	49·43	+0·86
14	55·82	11·99	42·47	54·46	+1·36
15	51·89	11·47	43·22	54·49	− 2·80
16	56·48	12·32	47·32	59·64	− 3·16
17	57·55	11·56	40·75	52·31	+5·24
18	63·30	13·43	48·49	61·92	+1·38
19	55·99	10·77	44·96	55·73	+0·26
20	44·72	8·40	42·92	51·32	− 6·60
21	42·52	7·87	38·70	46·56	− 4·04
22	44·97	8·97	36·21	45·18	− 0·21
23	37·33	8·44	31·92	40·36	− 3·03
24	45·22	7·97	39·25	47·22	− 2·00
25	44·48	8·95	34·71	43·66	+0·82

Table V. *Showing change of weight of Sheep III.*

Date	Weight of Sheep III	Date	Weight of Sheep III
May 10	8 st. 10 lb.	Aug. 2	8 st. 12½ lb.
June 2	8 „ 4 „	„ 17	8 „ 13 „
„ 20	8 „ 8 „	Sept. 3	9 „ 2½ „
July 4	8 „ 11 „	„ 19	9 „ 6 „

In discussing the nitrogen balance of Sheep III, the duration of the experiment may be conveniently divided into four periods, A, B, C

and D. During Period A, Sheep III was fed on a ration of cottonseed cake, barley meal and pasture grass. During Period B the ration was

Table VI. *Showing mean daily nitrogen balance of Sheep IV.*

Composite	Nitrogen consumed per day gm.	Nitrogen voided per day			Mean daily nitrogen balance gm.
		Faeces gm.	Urine gm.	Total gm.	
1	31·83	8·40	18·72	27·12	+4·71
2	—	Sheep scouring during this period			—
3	—		,,		—
4	20·85	7·63	20·43	28·06	− 7·21
5	35·82	7·97	20·09	28·06	+7·76
6	39·15	8·70	24·66	33·36	+5·79
7	38·04	9·51	28·12	37·63	+0·41
8	38·52	11·37	25·50	36·87	+1·65
9	43·63	11·26	30·02	41·28	+2·35
10	51·60	11·48	32·89	44·37	+7·23
11	51·84	21·96	38·08	60·04	− 8·20
12	61·58	12·81	41·01	53·82	+7·76
13	60·34	14·85	45·84	60·69	− 0·35
14	62·07	13·77	47·74	61·51	+0·56
15	55·48	11·55	38·44	49·99	+5·49
16	55·82	13·51	52·44	65·95	− 10·13
17	53·31	11·25	40·03	51·28	+2·03
18	59·19	13·02	54·23	67·25	− 8·06
19	47·32	11·56	47·01	58·57	− 11·25
20	45·82	10·00	38·14	48·14	− 2·32
21	40·38	8·50	35·74	44·24	− 3·86
22	37·85	7·27	38·29	45·56	− 7·71
23	40·61	7·64	33·84	41·48	− 0·97
24	42·71	8·08	34·55	42·63	+0·08
25	48·32	7·87	38·33	46·20	+2·12
26	44·22	6·86	37·46	44·32	− 0·10
27	44·29	7·34	38·83	46·17	− 1·88
28	51·76	8·52	41·95	50·47	+1·29
29	58·97	9·54	47·47	57·01	+1·96
30	60·63	10·88	49·33	60·21	+0·42
31	63·68	10·80	47·70	57·50	+6·18
32	48·35	8·91	44·52	53·43	− 5·08
33	48·62	8·78	40·58	49·36	− 0·74
34	49·56	10·65	37·17	47·82	+1·74
35	53·13	10·62	38·29	48·91	+4·22
36	58·48	9·43	36·51	45·94	+12·54
37	49·25	9·80	31·66	41·46	+7·79
38	54·61	9·34	39·98	49·32	+5·29
39	53·84	9·84	39·72	49·56	+4·28
40	50·82	8·68	37·31	45·99	+4·83
41	52·00	9·15	37·57	46·72	+5·28
42	50·16	7·45	45·89	53·34	− 3·18
43	56·24	9·96	39·16	49·12	+7·12
44	51·84	8·65	43·64	52·19	− 0·35
45	44·06	6·77	40·53	47·30	− 3·24
46	17·69	4·47	22·54	27·01	− 9·32
47	12·46	2·46	16·55	19·01	− 6·55
48	34·06	1·93	25·33	27·26	+6·80
49	38·49	4·36	28·83	33·19	+5·30
50	42·49	4·85	27·67	32·52	+9·97
51	10·41	1·75	10·06	11·81	− 1·40
52	7·95	1·18	9·74	10·92	− 2·97

changed to linseed cake, barley meal and pasture grass. During Period C the barley meal was eliminated from the ration and during Period D only linseed cake was fed. The initial weight of the animal was 8 st. 10 lb.

Table VII. *Showing change of weight of Sheep IV during experiment.*

Date	Weight of Sheep IV	Date	Weight of Sheep IV
May 10	8 st.	Sept. 19	9 st. 3 lb.
June 2	8 „	Oct. 3	9 „ 13 „
„ 20	8 „ 10 lb.	„ 13	10 „ ½ „
July 4	8 „ 12 „	Nov. 17	11 „ 3 „
Aug. 2	9 „	Dec. 7	11 „ 6 „
„ 17	9 „	„ 22	11 „ 3 „
Sept. 3	9 „ 1½ „	Jan. 12	10 „ 8 „

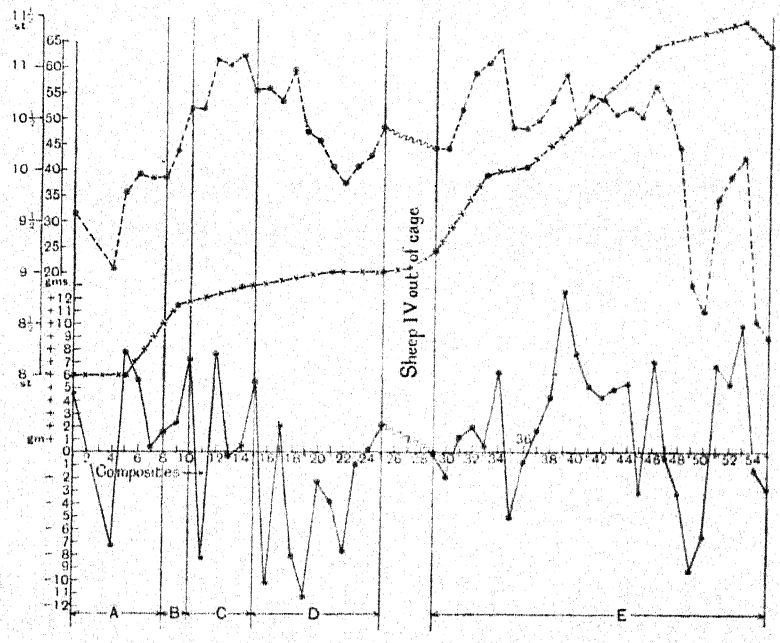


Fig. 4 shows Tables VI and VII in graphical form.

-------- Nitrogen consumption ×-×-× Change of weight
——— Nitrogen balance

During the first four composites, the animal scoured badly, the amount of food consumed decreased and it lost 6 lb. in weight. This resulted in a negative balance during this period. It is probable that the restraint of the cage was responsible for this behaviour. A return

to normal conditions was manifested by an immediate and sudden rise to a positive balance of 12 gm. accompanied by a gradual increase in weight. After this large rise in retention the animal during the rest of Period A and during B and C tends to come into nitrogenous equilibrium and during the last fortnight of Period C is actually in a state bordering on equilibrium. The fall to equilibrium takes place despite a rapid rise in consumption, but with only a small gain in weight.

During Period D, from the time it had a ration of linseed cake, the nitrogen balance is definitely negative. This is all the more remarkable since the animal reached its peak of nitrogen intake and showed its greatest weight during this period. Although there is a fall in nitrogen consumption once the maximum is reached, it must be appreciated that the consumption of protein is still much above the normal and that while some slight parallelism with the intake curve might be expected, a negative balance at this point is astonishing. Neglecting pasture grass, Sheep III consumed 66,000 gm. (145 lb.) of linseed cake and showed a gain of only 5½ lb. during Periods C and D, despite the fact that it was a young and immature animal and would be expected to derive the full amount of benefit from the protein digested. During Period D the digestibility coefficient of the protein ranged from 83 to 85 per cent., so it is obvious that whilst the animal was actually digesting this large amount of protein, it was not using it for body building purposes.

For the sake of convenience of discussion, it is proposed to divide the course of experiment of Sheep IV into four periods, A, B, C and D, which correspond exactly to those of Sheep III, and a fifth period, E, during which Sheep IV was still on a diet of linseed cake.

During Periods A, B, C and D, Sheep IV behaved similarly to Sheep III. It showed, during Period A, the negative balance due to cage conditions, but this time unaccompanied by loss of weight. This was followed by a sharp regain of protein by a rise to a positive balance of 7 gm.

This is not such a big rise as in the case of Sheep III and is probably accounted for by the fact that there was no loss of weight by Sheep IV. From the point of the sharp positive balance there was the same gradual tendency through Periods B and C, to come to equilibrium. This is not so obvious as in the case of Sheep III, being marked by more variations and more sudden rises and falls from high positive to high negative balances. During Period C the maximum nitrogen consumption was recorded, and, for this period taken as a whole, there was a slight positive balance. During Period D the protein consumption fell off to some

extent, but still remained at a high level. A very high negative balance was observed during the whole of Period D. The animal gained only 2 lb. in weight, despite the fact that it consumed 50,000 gm. (116 lb.) of linseed cake. After Period D both Sheep III and Sheep IV were allowed out of the metabolism cages for a period of 1 month. During this time their ration still consisted of linseed cake, and the daily consumption was accurately determined. At the end of the month both sheep showed a gain in weight, $10\frac{1}{2}$ lb. in the case of Sheep III and $6\frac{1}{2}$ lb. by Sheep IV. Sheep III was killed for pathological examination and Sheep IV was continued on experiment.

During Period E of Sheep IV's experimental life, which covered $3\frac{1}{2}$ months, a remarkable change took place in the protein metabolism. The animal showed no bad effects as a result of returning to the cage and ate its ration extremely well. It increased its protein consumption to a high figure, at which it remained constant for over 2 months. The animal then seemed to tire of its ration and its consumption fell off rapidly, decreasing in 10 days from 260 gm. of protein to approximately 20 gm. It recovered slightly, but not to any great extent, and was taken off experiment. During Period E there was a definite positive balance and, what was more remarkable, considering the increase in weight during Period D, a further sudden rise took place, from 9 st. 3 lb. to 11 st. $6\frac{1}{2}$ lb., an increase of 2 st. $3\frac{1}{2}$ lb. for a consumption of 234 lb. linseed cake. Following this the consumption fell off rapidly and a negative balance was again recorded. Corresponding to the fall in consumption and balance, however, there was an increase in weight of $3\frac{1}{2}$ lb.

The results during Period E proved most interesting. During Periods A, B, C, and D, Sheep III and Sheep IV show a remarkable parallelism as regards their nitrogen balances and their live weight increase; during Periods C and D, when their ration was for the greater part linseed cake, Sheep III consumed 73,000 gm. (161 lb.), showed a weight increase of $5\frac{1}{2}$ lb. and an average daily negative balance of 0·2 gm. Sheep IV consumed 74,000 gm. (163 lb.), showed a weight increase of $2\frac{1}{2}$ lb. and an average daily negative balance of 1·73 gm. During Period E, however, Sheep IV showed a positive balance, a weight increase of over 2 st., whilst its average consumption was almost identical to that of Period D. It would seem from these figures, that in order to reconcile the results of Periods D and E, the metabolism of the animal underwent some extraordinary change at the end of Period D.

The duration of the experiment on Sheep V may be divided very

conveniently into three periods, each of 1 month. Period A when its ration consisted of chaff, sugar beet pulp and linseed cake, Period B when the ration was sugar beet pulp and linseed cake, and Period C when the ration consisted entirely of linseed cake.

Table VIII. *Showing mean daily nitrogen balance for Sheep V.*

Composite	Nitrogen consumed per day gm.	Nitrogen voided per day			Mean daily nitrogen balance gm.
		Faeces gm.	Urine gm.	Total gm.	
1	39·12	9·90	25·19	35·09	+4·03
2	45·38	10·57	29·22	39·79	+5·59
3	46·49	10·66	32·15	42·81	+3·68
4	40·27	9·58	29·15	38·73	+1·54
5	36·49	9·06	26·35	35·41	+1·08
6	34·31	7·94	23·50	31·44	+2·87
7	34·38	7·29	21·62	28·91	+5·47
8	38·25	4·49	22·04	30·53	+7·72
9	34·60	6·50	22·55	29·05	+5·55
10	39·15	8·55	26·93	35·48	+3·67
11	42·77	8·60	29·37	37·97	+4·80
12	46·73	8·77	30·36	39·13	+7·60
13	43·15	8·44	27·31	35·75	+7·40
14	42·69	6·81	28·48	35·29	+7·40
15	40·80	7·77	27·55	35·22	+5·48
16	40·05	7·81	26·35	34·16	+5·89
17	41·08	6·41	30·00	36·41	+4·67
18	44·39	8·45	38·43	46·88	− 2·49
19	40·14	7·46	33·79	41·25	− 1·11
20	42·24	9·27	36·87	46·14	− 3·90
21	34·50	7·77	25·33	33·10	+1·40
22	43·20	7·13	30·11	37·24	+5·96
23	43·35	7·74	28·33	36·07	+7·28

Table IX. *Showing change of weight of Sheep V during experiment.*

Date	Weight of Sheep V	Date	Weight of Sheep V
Sept. 21	6 st. 7 lb.	Dec. 22	7 st. 9 lb.
Oct. 13	6 „ 6½ „	Jan. 12	6 „ 9 „
Nov. 17	7 „ 1 „		

During Period A there is seen the usual result of the animal becoming accustomed to cage conditions, the falling consumption and retention of protein. This is followed by a sharp rise to a high positive balance no doubt due to the animal regaining condition. Sheep V did not show the tendency exhibited by Sheep III and Sheep IV to quickly assume a state of nitrogenous equilibrium, but maintained a definite positive retention of 5 or 6 gm., which coincided with a high protein consumption. This may be accounted for by the very immature state of the animal which was only 7 months old when put on experiment. During Period C, when the sugar beet pulp was eliminated from the ration, there was a fall to a negative balance. In the case of Sheep V the fall was very

sharp. No gradual fall to equilibrium was obtained, but a sudden change was made from a high positive to a negative balance. The negative balance was maintained during the whole of Period C. The consumption of protein remained at a constant high figure throughout this period, so that there is no parallelism in this instance between intake and retention.

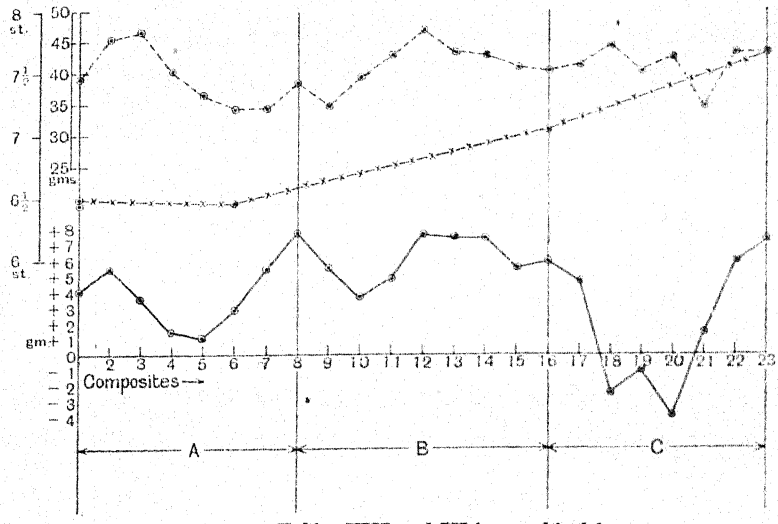


Fig. 5 showing Tables VIII and IX in graphical form.

- - - - - - - - Nitrogen consumption ×-×-× Change of weight
———— Nitrogen balance

Sheep V showed a constant, but very gradual, change of weight. During Period B the weight increased from 6 st. 6½ lb. to 7 st. 1 lb. and to 7 st. 9 lb. during Period C. The 8 lb. increase in weight in Period C corresponded to a consumption of approximately 25,000 gm. (55 lb.) of linseed cake. This was a slightly larger gain in weight than registered by Sheep III or Sheep IV during Period D, but very small in proportion to the ration fed.

Discussion on nitrogen balances.

One of the most important results of the present investigation is the evidence which has been produced regarding the unreliability of short period balance experiments, and the great care that is necessary in the interpretation of their results. This is seen most strikingly in the initial effects which the metabolism cage has upon the animal. Every

animal showed a fall in nitrogen consumption and a negative balance out of all proportion to the fall in consumption usually accompanied by loss of weight during the first month on experiment. Undoubtedly this is due to the change in living conditions: a change usually from the open field to the confined space of the cage, which entails a loss of exercise and frequently a marked change of diet. If an animal takes 4 weeks to settle down to its new environment (as has been shown in the present investigation), then only after 4 weeks can it be said to show a normal metabolism. Consequently in any balance experiment 6 weeks should be the minimum length of the pre-period which is to show the normal metabolism of the animal. This point cannot be too strongly emphasised, since many research workers both in this country and in America use only the first 3 weeks during which an animal is in the metabolism cage as a pre-period. This being the case any changes in metabolism which are recorded in their experimental period must be regarded with suspicion, since it may well be that these changes are only due to the animal returning to the normal from the initial effects of cage restraint. Another difficulty in short period balance work shown by the results detailed above is the interpretation of the sudden jumps from a high positive to a high negative balance in the course of a few days. These sudden changes, usually inexplicable, occurring in the middle of a long period balance experiment are not of much importance in affecting the general significance of the results. When occurring in short period balance work, however, besides being inexplicable they are also entirely misleading and often tend to make the work valueless since they cannot be disregarded, the total length of the experimental period being so short. Having regard to the above facts, care must be exercised in accepting the conclusions drawn from short period balance experiments.

Sheep I behaved normally, and as previously stated gave results similar to those obtained by Crowther and Woodman (4), who found that above the maximum nitrogen intake point which coincided with the optimum nitrogen point the excess protein ingested was not used for body building and the animal proceeded to a state of nitrogenous equilibrium. That Sheep I did not proceed to a state of nitrogenous equilibrium but maintained a constant retention despite an increasing consumption may well be due to the fact that Sheep I was an immature animal, whilst those used by Crowther and Woodman were mature, and would naturally only be retaining protein to repair the tissue broken down in the normal course of metabolism.

The rations fed to Sheep III, IV and V were initially even higher in protein than that fed to Sheep I, and therefore probably above the maximum point for optimum retention, which may account for the fact that these animals proceeded to a state of nitrogenous equilibrium immediately they had regained their normal condition after the first effects of the cage had worn off. Since Sheep V was the youngest on experiment, it would be expected to make more use of the protein ingested and would therefore be considerably longer in reaching a state of equilibrium. Though stress has been laid on the fact that Sheep V was a very young animal, it must not be forgotten in discussing the balances, that all the animals were really immature, and would be expected to be body building during the period they were on experiment.

The most surprising feature of the balance curves is the complete unanimity with which, during the first 3 or 4 months on experiment, Sheep III, IV and V tend to show first a nitrogenous equilibrium and then a negative balance. The agreement on this point between the three experiments shows that it cannot be a singular coincidence, but must be due to some important factor in the metabolism of a high and excessive protein diet.

Protein has a twofold function in metabolism. A certain proportion is used for the repair of the wear and tear of the tissues. The amino acids into which it is resolved in the intestines circulate in the blood and each living cell picks out those particular amino acids which are essential to building up the protoplasm of that cell. The rest of the protein loses its nitrogen and on deamination is used for energy and the production of fat.

What then has happened to the protein of the excessive amount of linseed cake consumed by Sheep III, IV and V? It has not caused any increase of fat or muscle in the body, since no significant change in weight was observed during the 4 months. It has not even prevented loss of protein from the body, since a negative balance was evinced despite the fact that the protein of the cake had been digested as seen from the increase in urinary nitrogen. From this it must be supposed that all the protein has been entirely deaminated, and therefore all the nitrogen has been lost as urea. As has been explained above, however, the deaminated amino acids should in part be oxidised for the production of heat and energy, and the surplus should be converted into fat. Since no increase in weight was observed, which leads to the belief that all the protein was deaminated, and if deaminated not used for fat production, all the deaminated amino acids must have been oxidised and

used for heat, but one would have expected the whole of such acids to be used as fat, since linseed cake contains sufficient oil and carbohydrate for energy and heat purposes, especially as the animals were in confinement and had no wastage of energy in exercise.

This huge production of heat and energy may partly be explained by Rubner's specific dynamic theory of protein metabolism. Rubner found that an increase of protein to the diet of an animal increased its metabolism. This is explained by stating that protein has an excitant action on the processes of oxidation occurring in the body by virtue of which an animal will give out more heat and consume more food on a protein diet than on a diet of the same calorific power but in which carbohydrates preponderate.

Though the specific dynamic theory may be accepted as part explanation of the above, it is beyond doubt not the whole truth of the extraordinary result, whereby the excessive amount of protein ingested was used for neither tissue nor fat production but wasted instead entirely in the production of heat, since even the specific dynamic action of protein results only in the production of heat and cannot be utilised for the performance of muscular work. Moreover, it has been proved in the case of immature animals that the specific dynamic action of protein is at a minimum, since the great portion of protein ingested by these animals is necessary for body-forming purposes, and so in the results of the present investigation too great stress cannot be laid on Rubner's theory, as a satisfactory explanation of the big negative balances recorded.

The results mentioned above are further complicated when Period E of Sheep IV's experimental life is considered. During Period E, Sheep IV showed a normal metabolism or rather one which followed the general accepted theories of linseed cake metabolism. The animal with practically the same consumption as in Section D showed a change to a definite positive balance, and a big increase in weight. It is apparent from this that a sudden change took place in the metabolism of the protein. That this is not due to a change in the protein itself is at once evident by the fact that linseed cake was fed throughout Sections D and E, and that even the same quality was fed over the transition stage from D to E. A simple solution of this extraordinary change may be as follows. The animal had been on experiment for 4 months and may perhaps by virtue of necessity had to readjust its metabolic processes to make use of the breakdown products of the protein offered it, though these breakdown products may not have been the ones accus-

tomed to be used for body-building purposes, or it may be that by constant feeding of the ration of linseed cake the animal was able to use the protein in an earlier state of breakdown, instead of in the amino acid state in which they are usually absorbed in the blood. Either of the above suppositions would entail too great an upheaval in the physiological processes of the animal to be considered possible, and in this most unsatisfactory condition must it be left, since only a complete analysis of both blood and urine would have shed any light on a drastic change in the physiology of the animal's metabolism.

Another consideration must be taken into account when the results are reviewed. It must be remembered that the source of the high protein diet was linseed cake, and it may be that it is owing to the peculiar properties of the protein of linseed cake that the results in the present investigation were obtained. If this view is held, it leads to the supposition that the protein of linseed cake is of very small metabolic efficiency, or biological value, since no weight increase was observed over the first 4 months of the experiment, and thereby linseed cake must be considered a most uneconomic foodstuff. If this is held to be the correct interpretation of the results of the present experiments, *i.e.* that the amino acids derived from linseed cake are not the essential ones required for tissue building and repairing, then the solution of the problem as to why no fat was produced on deamination has still to be explained. It must also be borne in mind that Kellner, by accurate experiments carried out in a calorimeter where heat production was recorded as well as excreta, etc. being analysed, found the metabolism of linseed cake to be quite normal. Also Foreman(5) has shown that linseed cake contains most of those essential amino acids in good proportions. Therefore it must be concluded that the proteins of linseed cake are of high biological value.

In this inconclusive state must be left the interpretation of the surprising balances of the animals on high protein diet, since the data at hand are insufficient to enable a complete explanation to be formulated. The results obtained, however, are of sufficient importance to show the need of a very close enquiry into the physiology of protein metabolism during high protein feeding.

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(*Received June 1st*, 1929.)

THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS, VI.

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The application of sulphur in spray form is often advantageous in that the incorporation in the spray of a contact insecticide, such as nicotine, is then possible. In this way, for example, the operation of dusting hops against "mould" and of washing against "aphis" can be accomplished simultaneously with obvious saving to the hop-grower. The use of soluble mildew-killing materials such as belong to the polysulphide group is not always possible because of injury to the plant. An examination has therefore been made of the various methods of using elementary sulphur in spray form and of the efficacy of such sprays against the hop "powdery" mildew (*Sphaerotheca Humuli* (DC.) Burr.). For the latter purpose, the methods evolved for the exact comparison of the fungicidal properties of spray-fluids, the technique of which has already been described in previous communications to this *Journal* (1, 2, 5, 7), was used.

The methods of using elementary sulphur in spray form fall into two groups: firstly, the use of ordinary dusting sulphurs treated to enable their incorporation in the spray and, secondly, the employment of so-called colloidal sulphurs either home-prepared by the interaction of certain sulphur compounds whereby sulphur is precipitated in a sprayable form, or commercial preparations in which elementary sulphur is already present in a finely-divided and wetted form. Accordingly the experiments recorded below fall into the classes (1) "wetted" sulphurs, and (2) colloidal sulphurs.

I. "Wetted" sulphurs.

The problem of securing a wetting of the leaf or fungus surface is, in practice, usually solved by the addition to the spray of a "spreader." By the same means it is possible to secure a wetting of the sulphur particle. Robertson (8), in 1821, used soap suds for this purpose and the majority of the capillary active materials employed in spraying practice as spreaders have since been recommended from time to time as sulphur wetters. It is difficult, however, to break up the agglomerates of sulphur which readily form, especially in ground sulphurs, and which would

interfere with the successful distribution of the spray. Although it is possible to break down most of the lumps by prolonged agitation, the operation is sufficiently troublesome in practice to warrant attempts at its elimination.

One method, recommended by Vermorel and Dantony (11), involves a pre-treatment of the sulphur with an alcoholic solution of oleic acid. Alcohol alone serves admirably to break down the agglomerates to "prime particle structure" and the disadvantage of its ready volatility is overcome by the addition of oleic acid which, remaining on the particles, prevents the re-agglomeration. A second method involves the incorporation with the sulphur of an inert powder, such as kaolin or hydrated lime, by which means the "balling-together" of the sulphur particles is prevented. The exact nature of the action of the inert material is unknown, but Streeter (9) considered it due to a neutralisation of the electric charge generated upon the sulphur particle by friction. A successful example of this latter type of sulphur preparation is the "Dry-Mix Sulphur Lime" recommended by Farley (3).

The fungicidal activity of the sulphur-soft soap spray.

The first experiments to be described deal with the fungicidal properties of different forms of sulphur applied by using solutions of soft soap as the wetter-spreader. The samples of sulphur used included a typical flowers of sulphur (Brandrams) and two ground sulphurs, Fortress brand and Monro's dry dusting sulphur. The samples were all of a high degree of purity and an estimate of the relative degrees of fineness[1] of the two ground sulphurs showed little difference.

In certain cases the sulphurs were treated with oleic acid (100 gm. of sulphur were moistened with 4 c.c. methylated spirit and 1 c.c. oleic acid) prior to mixing with the soft soap solution, whilst in other experiments soft soap alone was used. The suspensions so obtained were

[1] To compare the relative degrees of fineness of the ground sulphurs, a method based upon the mechanical analysis of soils was employed. Weighed amounts of sulphur were shaken with a measured volume of a mixture of 1 part medicinal paraffin and 2 parts of ordinary paraffin in an end-over-end shaker for 30 minutes to break up the aggregates of sulphur. The mixture was poured into a wide cylinder, and, after standing for various periods of time, aliquots were removed from certain depths by means of a pipette. Unfortunately it was found impossible to secure concordant results under varying conditions, perhaps because of the large correction necessary for viscosity changes due to temperature. Simultaneously conducted trials, however, failed to reveal any large consistent differences between the amounts of sulphur separating at the same rates from the two samples, and it is permissible to conclude that there was no great difference between the relative degrees of fineness of the two samples.

sprayed by means of a hand "atomiser" and no serious difficulty was encountered in getting the suspension to pass the extremely fine aperture of the nozzle. Despite vigorous shaking, however, it was found that much sulphur remained at the bottom of the container. It was found impossible to arrive by subtraction at an accurate figure for the amount of sulphur applied, for the conidiophores of the fungus acted as a filter and concentrated the sulphur upon the fungus. The figure given for the initial concentration of sulphur employed therefore does not accurately indicate the concentration of sulphur actually applied to the mildew. It may be assumed, however, that as the various sulphurs differ but little in degree of fineness, similar concentrations of sulphur were applied when similar initial concentrations of sulphur were used.

Exp. A 14. 2 gm. treated[1] ground sulphur (Fortress) in 100 c.c. of 1 per cent. soft soap solution.

Five leaves were sprayed. The action was fungicidal for the older patches but not quite fungicidal for the younger, a few clustered conidiophores re-growing from their edges.

Exp. A 20. 5 gm. treated ground sulphur (Monro) in 100 c.c. of 1 per cent. soft soap solution.

Eight leaves were sprayed. Complete fungicidal action was obtained.

Exp. A 23. 5 gm. treated flowers of sulphur (Brandrams) in 100 c.c. of 1 per cent. soft soap solution.

Nine leaves were sprayed. Complete fungicidal action was obtained.

Exp. A 22. 2 c.c. of the alcohol-oleic acid mixture used for treating the sulphur in 100 c.c. of 1 per cent. soft soap solution.

Two leaves were sprayed. The solution proved non-fungicidal, the mildew patches being densely powdery again by the fourth day.

Exp. A 16. 5 gm. treated ground sulphur (Monro) in 100 c.c. of 1 per cent. soft soap solution.

Seven leaves were sprayed. The action was fungicidal for all the patches.

As experience showed that sulphur, both ground and flowers, could be effectively wetted by means of a solution of soft soap alone, the use of alcohol and oleic acid was abandoned in the experiments of series B and C which follow.

Exp. B 2*. 3 gm. ground sulphur (Monro) in 100 c.c. of 0·5 per cent. soft soap solution.

[1] The word "treated," when used, indicates that the sulphur had been moistened with alcohol and oleic acid, in the manner already described, before being mixed with the soft soap solution.

Exp. B 3*. 4 gm. ground sulphur (Monro) in 100 c.c. of 0·5 per cent. soft soap solution[1].

Seven leaves were sprayed. No difference was observable in the fungicidal action of the two suspensions, and practically all the conidiophores of the powdery patches were collapsed on the day after application. By the ninth day, except for some isolated weak clusters of conidiophores at the edge of a few patches, the mildew patches were killed.

Exp. B 17*. 3 gm. ground sulphur (Monro) in 100 c.c. of 0·5 per cent. soft soap solution.

Exp. B 18*. 1 gm. ground sulphur (Monro) in 100 c.c. of 0·5 per cent. soft soap solution.

Eleven leaves were sprayed. The 3 gm. per 100 c.c. suspension proved fungicidal on ten of the leaves, on the eleventh leaf most of the patches were killed, but a few weak clusters of conidiophores developed in the hollows of the veins of the leaf or at the edge of a few patches. The 1 gm. per 100 c.c. suspension proved fungicidal on eight leaves, but on three leaves a few conidiophores developed by the ninth day on the edges of a few of the patches, the remainder being killed.

Exp. B 33*. 2 gm. ground sulphur (Monro) in 100 c.c. 0·5 per cent. soft soap solution.

Exp. B 34*. 2 gm. flowers of sulphur (Brandrams) in 100 c.c. of 0·5 per cent. soft soap solution.

Ten leaves were sprayed. In neither case did the suspensions prove quite fungicidal. On seven leaves in both experiments, while many patches remained sterile and died, several produced small clusters of conidiophores at the centre or edge and on three of the leaves most of the patches re-formed vigorous clustered conidiophores.

Exp. C 34. 5 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. soft soap solution.

Seven leaves were sprayed. The suspension proved fungicidal.

Exp. C 66. 1·92 gm.[2] ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. soft soap solution.

Eleven leaves were sprayed. The suspension proved fungicidal.

Exp. C 74. 1 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. soft soap solution.

[1] Where two consecutive experiments are marked with asterisks the first spray was applied to one of the leaves at a node and the other spray to the opposite leaf of the same node.

[2] This amount of sulphur is the same as that contained in the 3 per cent. dry-mix sulphur lime used in Exp. C 65 (see p. 25).

Eleven leaves were sprayed. The action was fungicidal on eight leaves and not quite fungicidal on three leaves—a few clusters of conidiophores being produced from some of the patches.

The above experiments with suspensions of sulphur in soft soap solutions clearly indicate the marked fungicidal action which they all possess. The amount of sulphur applied to the fungus may not be in proportion to the strength of the suspension, owing to variations such as settling of particles during spraying and retention of sulphur upon the mildew patches. In Exps. B 2 and B 3, where 3 gm. and 4 gm. sulphur per 100 c.c. respectively were compared, no difference in fungicidal action was observable. At the lower concentrations the fungicidal action of the sulphur proved variable, as shown in Exps. A 14, B 33 and B 34 in which 2 gm. sulphur per 100 c.c. was not quite fungicidal, whilst a suspension of 1·92 gm. per 100 c.c. (Exp. C 66) was fungicidal. Suspensions of 1 gm. per 100 c.c. had also a very considerable, but not quite complete, fungicidal action as seen in Exps. B 18 and C 74. In all the experiments where suspensions of 5 gm. of sulphur per 100 c.c. were used, complete fungicidal action was obtained.

The particular samples of sulphur used in the above experiments were found to have similar fungicidal properties when used at the same strengths and under the same conditions. In Exps. B 33 and B 34, flowers of sulphur was compared with ground sulphur, but no difference in effect on the fungus could be observed.

The influence of type of spreader upon the fungicidal activity of sulphur.

Attention was next directed to the influence of the spreader upon the fungicidal activity of the wetted sulphur. Reference has been made in a previous paper (6) to the marked superiority of sulphur applied with soft soap to sulphur applied as a dust, and there are grounds for believing that this superiority is not due solely to an improved dispersion of the sulphur upon the fungus. The theoretical considerations upon which this view is based are discussed in the paper which follows the present one.

For the purpose of this investigation, solutions of various wetting agents were prepared as follows:

Soft soap. A commercial potash soft soap containing neither free alkali nor resin; the strength employed was 0·5 per cent.

Gelatine. 0·5 per cent. solution, freshly prepared, of Coignet's No. 1, Fine.

Saponin. The commercial buff-coloured grade was employed at 0·5 per cent. strength.

Casein. Commercial "Light Yellow" containing 10·74 per cent. nitrogen. The various caseinate solutions used were prepared by dissolving the casein in solutions either of sodium or calcium hydroxides of known strength. The lime casein suspension used in Exp. A 13 was prepared by intimately mixing 25 gm. casein with 10 gm. calcium oxide; the mixture was stirred with 250 c.c. water for some time, then filtered through cloth and the solution so obtained, which was approximately 10 per cent., was diluted to give the 1 per cent. solution required. In Exp. C 58, the soda casein solution was prepared by dissolving 5 gm. casein in 25 c.c. *N* sodium hydroxide and diluting to 500 c.c.; and in Exp. C 71, 2·5 gm. casein was dissolved in the same amounts of sodium hydroxide and water. For Exp. C 49, casein was dissolved in a boiling suspension of calcium hydroxide, the mixture filtered when cold yielding a solution containing 0·136 gm. calcium oxide per 100 c.c.

Dry-mix sulphur lime. Farley (3) recommended the intimate mixing of 16 parts of dusting sulphur, 8 parts of hydrated lime and 1 part of casein spreader, the mixture being applied at the rate of 25 lb. per 100 U.S. gallons, which approximates to 30 lb. per 100 Imperial gallons. "Fortress" brand ground sulphur was used. The casein spreader used was the commercial lime casein "Kayso" which was found to approximate to a mixture of 1 part of casein and 3 parts of hydrated lime. The particular sample employed was, however, much carbonated and contained 44·4 per cent. calcium carbonate. The hydrated lime contained 69·36 per cent. total calcium oxide with only 2·52 per cent. calcium carbonate.

The suspensions of sulphur were prepared by stirring the sulphur in a mortar with a small amount of the spreader solution to a thin paste. The bulk of the spreader solution was then added and the mixture shaken 7 hours in an end-over-end shaker immediately before application to the hop leaf.

Exp. A 13*. 2 gm. ground sulphur (Fortress) in 100 c.c. of 1 per cent. solution of lime casein.

Exp. A 14*. 2 gm. treated ground sulphur (Fortress) in 100 c.c. of 1 per cent. soft soap solution.

Seven leaves were sprayed. The suspension proved non-fungicidal in Exp. A 13 and almost fungicidal in Exp. A 14 (see p. 20).

Exp. B 37*. 3 gm. ground sulphur (Monro) in 100 c.c. of 0·5 per cent. soft soap solution.

Exp. B 38*. 3 gm. ground sulphur (Monro) in 100 c.c. of 0·5 per cent. gelatine solution.

Nine leaves were sprayed. The suspension proved fungicidal in Exp. B 37; in Exp. B 38, the suspension proved unsatisfactory and little fungicidal action was obtained.

Exp. C 32*. 5 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. gelatine solution.

Exp. C 34*. 5 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. soft soap solution.

Seven leaves were sprayed. The suspension proved non-fungicidal in Exp. C 32, clustered conidiophores re-forming by the sixth day and all the patches being powdery again by the ninth day. In Exp. C 34, fungicidal action was obtained.

Exp. C 54*. 5 gm. ground sulphur in 100 c.c. of 0·5 per cent. gelatine solution.

Exp. C 53*. 5 gm. ground sulphur (previously washed[1]) in 100 c.c. of 0·5 per cent. gelatine solution.

Ten leaves were sprayed. The suspension proved non-fungicidal in both cases, all the patches being again powdery by the seventh day.

Exp. C 45*. 5 gm. ground sulphur (Fortress) plus 0·2 per cent. sodium hydroxide in 100 c.c. 0·5 per cent. saponin solution.

Exp. C 46*. 0·2 per cent. sodium hydroxide in 0·5 per cent. saponin solution.

Eight leaves were sprayed. Marked fungicidal action was obtained in Exp. C 45, many of the patches on all the leaves were killed, while others showed few or many scattered conidiophores arising from mycelium at their edges. In the control experiment, C 46, the spreader solution proved non-fungicidal.

Exp. C 50*. 5 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. saponin solution.

Exp. C 49*. 5 gm. ground sulphur (Fortress) in 100 c.c. of a solution of lime casein (containing 0·136 gm. CaO per 100 c.c.).

Fourteen leaves were sprayed. In neither case was the suspension fungicidal; conidiophores began to be re-formed by the third day and the patches were more or less powdery again by the tenth day. On some of the leaves in Exp. C 49 there were, however, fewer conidiophores than on the corresponding leaves in Exp. C 50.

[1] Fortress brand ground sulphur was employed, in one case the sulphur being washed with alkali prior to use. The sulphur was first wetted with alcoholic sodium hydroxide and washed with alcohol, a solution of Agral I and finally with the gelatine solution.

Exp. C 58*. 5 gm. ground sulphur (Fortress) in 100 c.c. of a soda casein solution.

Exp. C 57*. 7·8 gm. dry-mix sulphur lime (containing 5 gm. sulphur) in 100 c.c. of water.

Eleven leaves were sprayed. The suspension was fungicidal in Exp. C 57 but not quite fungicidal in Exp. C 58, for, although some of the patches were killed, many of them produced few or numerous conidiophores usually scattered and arising from the edge of the patch.

Exp. C 71. 5 gm. ground sulphur (Fortress) in 100 c.c. of a 0·5 per cent. soda casein solution.

Ten leaves were sprayed. On some of the leaves the action was fungicidal, all the patches being killed; on the remaining leaves (four) several patches re-formed scattered conidiophores at their margins.

Exp. C 65*. 3 gm. dry-mix sulphur lime (containing 1·92 gm. sulphur) in 100 c.c. water.

Exp. C 66*. 1·92 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. soft soap solution.

Eleven leaves were sprayed. In Exp. C 65 the fungicidal action was almost complete; all the patches were killed on seven leaves, while on the remaining four a few clustered conidiophores were re-formed at the edges of two or three patches, the remaining being killed. In Exp. C 66 the suspension proved fungicidal (see p. 21).

Exp. C 73*. 1·56 gm. dry-mix sulphur lime (containing 1 gm. sulphur) in 100 c.c. water.

Exp. C 74*. 1 gm. ground sulphur (Fortress) in 100 c.c. of 0·5 per cent. soft soap solution.

Eleven leaves were sprayed. The fungicidal action was almost complete in Exp. C 73, all the patches being killed on seven leaves, while a small number of conidiophores developed from a few patches on the remaining leaves. In Exp. C 74 the fungicidal action was again almost complete (see p. 21).

The above experiments indicate that the fungicidal action of sulphur, when applied with soft soap, is greater than when it is used with gelatine or saponin. Available evidence points to an inhibition of the hydrolysis of the sulphur by solutions of gelatine or saponin, probably owing to their acid nature. On the other hand, soft soap presumably promotes the fungicidal activity of sulphur by virtue of the alkalinity produced by the hydrolysis of the soap. With the casein spreaders, the excess of alkali favours the fungicidal action of the spray. This is shown in Exp. C 49, where the excess of calcium hydroxide was small and fungi-

cidal action was not as complete as in Exp. C 57, where a large amount of hydrated lime was used. Similarly, fungicidal action does not appear to be as complete in Exp. C 58 as in Exp. C 71. The soda casein used in Exp. C 58 contained a larger proportion of casein which would reduce the alkalinity of the mixture.

In Exps. C 65 and C 66, C 73 and C 74, the action of sulphur and soft soap was compared with the action of sulphur in dry-mix sulphur lime. The sulphur-soft soap mixture proved to be the better fungicide in the case of Exp. C 66, whereas little difference could be detected in the action of sulphur in Exps. C 73 and C 74. In this connection it may be noted that the dry-mix sulphur lime, at concentrations lower than 3 gm. per 100 c.c., did not wet the fungus as satisfactorily as did the sulphur-soft soap mixture.

II. Colloidal sulphurs.

Within the last few years a number of commercial preparations containing sulphur in an extremely finely-divided form—so-called "colloidal" sulphurs—have been placed on the market. It was claimed that sulphur in such forms would prove sufficiently potent a fungicide to permit economies despite the higher initial cost of such products. They were prepared either by mechanical means—the grinding of sulphur in the presence of a protective colloid or "stabiliser" in mills of the Plauson type, or by chemical means—the precipitation of sulphur from polysulphide compounds or from alkali thiosulphate solutions by the addition of acid in the presence of a protective colloid. In the latter case, however, the material, as with the home-made colloidal sulphurs (12), may contain much sulphur in forms other than the elementary sulphur whose fungicidal activity was to be tested. In the former case, the majority of the products were found to contain much organic matter.

Many of the commercial products seemed to be unsuited to practical use owing to difficulties in obtaining a satisfactory suspension under field conditions. Thorough trials were therefore limited to one commercial colloidal sulphur marketed under the name "Ialine." This material was found to contain approximately 50 per cent. elementary sulphur, to be almost neutral in reaction and to be free from impurities other than a small amount of sulphur in the form of sulphate. A number of samples obtained from various sources were employed for the extended trials recorded below. Analysis showed the various samples to be of approximately the same composition.

For the determination of the sulphur present in elementary form, Chapin's method(4) was adopted, the percentage of "free" sulphur being obtained by an estimation of the amount of sodium thiosulphate formed when an aliquot of the suspension was heated upon a boiling water bath with excess of sodium sulphite in the presence of ammoniacal zinc chloride solution.

Spray trials were carried out to determine the minimum strength necessary for fungicidal action, soft soap (recommended by the manufacturers) being used as the spreader. Table I gives the results obtained (F. = fungicidal):

Table I.

No. of exp.	%	Soft soap %	No. of leaves sprayed	Effect on fungus
A 39	0·2	1	6	F. for older patches only
A 41	0·2	0·5	9	F.
B 3	0·4	1	4	F.
B 4	0·2	1	4	F. or nearly F.
C 1	0·2	0·5	7	Non-F.
C 2	0·3	0·5	6	Non-F.
C 3	0·4	0·5	7	Not quite F.
C 17	0·4	0·5	7	Not quite F.
C 5	0·8	0·5	5	Almost F.
D 39	0·1	1	11	F.
D 35	0·2	1	10	F.
D 42	0·2	0·5	9	F.
D 18	0·3	1	8	F.
D 19	0·3	1	10	F.
D 11	0·4	1	13	F.
D 25	0·4	1	12	F.
D 6	0·5	1	4	F.
D 26	0·5	1	9	F.
E 40	0·1	0·5	8	Not quite F. to non-F.
E 77	0·1	0·5	7	Not quite F. to non-F.
E 39	0·2	0·5	8	F. or nearly F.
E 59	0·2	0·5	10	F. or nearly F.
E 60	0·3	0·5	10	F.
E 25	0·2	0·5	10	F.
E 22	0·4	0·5	10	F.
E 41	0·4	0·5*	10	F.
E 42	0·4	0·5	10	F.

* In this experiment hard soap was employed in place of soft soap.

The above results show that at a rate of 2 lb. per 100 gallons (0·2 gm. per 100 c.c.) the suspensions were fungicidal or nearly fungicidal. At 0·1 gm. per 100 c.c. the results were recorded as not quite fungicidal to non-fungicidal, with the exception of Exp. D 39, where the action was fungicidal. It will be noted that in series D the soft soap was used generally at 1 per cent. With suspensions of 0·3 gm. or more per 100 c.c. the suspensions were uniformly fungicidal. The C series is, however, anomalous, as in all the experiments in that year, the results, for some

reason which at the moment it is impossible to explain, were abnormal. The experiments in the last series (E) may be regarded as typical and to indicate that, for the hop powdery mildew, this particular form of colloidal sulphur when used with 0·5 per cent. soft soap (5 lb. per 100 gallons) is fungicidal at strengths of 3 lb. per 100 gallons and beyond. In Exps. E 41 and E 42 a comparison was made between hard soap and soft soap but no difference in effect was observed.

The striking difference between the amounts of colloidal sulphur and wetted ground sulphur which are required for complete fungicidal action points to fineness of division being a very important factor in the efficiency of sulphur as a fungicide. In this connection it may be noted that in a 0·3 gm. per 100 c.c. suspension of colloidal sulphur there was 0·157 gm. "free" sulphur per 100 c.c. (Exp. E 60), an amount which approximates in order to the quantity of polysulphide sulphur found to be fungicidal to the hop powdery mildew in the case of the polysulphide sprays used with soft soap.

The influence of spreader upon the fungicidal activity of colloidal sulphur.

Reference has been made in the section (I) dealing with wetted sulphurs to the influence of the spreader upon the fungicidal action of sulphur, and the same question arises with colloidal sulphur. The same spreaders were used as in the case of ground sulphur and, in addition, experiments were made in which "Agral I" was employed. Agral I is a sulphonated aromatic derivative introduced by the British Dyestuffs Corporation under the name "Perminal" as a wetting agent in textile processing and employed, under the name "Agral I," by Tutin[10] as an emulsifier. The substance is readily soluble in water to give a solution possessing excellent spreading and wetting properties. Its comparative non-reactivity and the approximate neutrality of its aqueous solution renders it of particular value in experimental work.

It soon became evident that when gelatine or saponin was used in place of soft soap an entirely different effect was produced. This point is shown by the following comparative trials:

Exp. E 5*. 2 per cent. colloidal sulphur plus 0·5 per cent. soft soap solution.

Exp. E 6*. 2 per cent. colloidal sulphur plus 0·5 per cent. gelatine solution.

Eight leaves were sprayed. In Exp. E 5 fungicidal action was

obtained. In Exp. E 6 the suspension proved to be non-fungicidal; new, densely-clustered conidiophores were present by the fifth day and by the eighth day all the patches were densely powdery.

Exp. E 13*. 4 per cent. colloidal sulphur plus 0·5 per cent. soft soap solution.

Exp. E 14*. 4 per cent. colloidal sulphur plus 0·5 per cent. gelatine solution.

Eleven leaves were sprayed. The colloidal sulphur with soft soap proved fungicidal. With gelatine the colloidal sulphur had a quite different action; on two of the leaves only were the patches sterile; on six leaves the older patches were killed but scattered conidiophores were formed at the edges of the younger patches; on three leaves most of the patches had re-formed clustered conidiophores, powdery with conidia, by the tenth day.

Exp. E 38*. 1 per cent. colloidal sulphur plus 0·5 per cent. saponin.

Exp. E 37*. 1 per cent. colloidal sulphur plus 0·5 per cent. soft soap.

Ten leaves were sprayed. Complete fungicidal action was obtained in Exp. E 37. In Exp. E 38 the suspension was non-fungicidal, the mildew patches bearing clustered conidiophores by the sixth day and being densely powdery by the tenth day.

The above results agree with those found in the case of wettable sulphur. To test the hypothesis that the acid nature of the solutions of gelatine and saponin was responsible for the difference in behaviour of these spreaders as compared with soft soap, a number of experiments were carried out in which the gelatine solution was made alkaline by the addition of caustic soda. The following may be quoted:

Exp. E 28*. 2 per cent. colloidal sulphur plus 0·5 per cent. gelatine.

Exp. E 29*. 2 per cent. colloidal sulphur plus 0·5 per cent. gelatine plus 0·024 per cent. caustic soda.

Nine leaves were sprayed. In both the experiments the action was non-fungicidal.

Exp. E 35*. 2 per cent. colloidal sulphur plus 0·5 per cent. gelatine plus 0·15 per cent. caustic soda.

Exp. E 36*. 0·5 per cent. gelatine plus 0·15 per cent. caustic soda.

Six leaves were sprayed. In Exp. E 35 the mixture proved fungicidal on some leaves and almost fungicidal on others; in Exp. E 36, the patches were densely powdery again by the tenth day.

Exp. E 43*. 2 per cent. colloidal sulphur plus 0·5 per cent. gelatine plus 0·2 per cent. caustic soda.

Exp. E 44*. 0·5 per cent. gelatine plus 0·2 per cent. caustic soda.

Ten leaves were sprayed. By the tenth day the patches on all the leaves in Exp. E 43 bore conidiophores—usually more or less scattered and at the edges. It was clear that the mixture was almost but not quite fungicidal. In Exp. E 44 the action was non-fungicidal.

It will be seen that the addition of a small amount of caustic soda (0·024 per cent.) in Exp. E 29 had no effect upon the fungicidal action of the colloidal sulphur. A larger amount of caustic soda, 0·15 per cent. (Exp. E 35), resulted in the mixture being fungicidal on some leaves and almost fungicidal on others. In Exp. E 43, where 0·2 per cent. caustic soda was used, the mixture proved to be almost but not quite fungicidal. Exps. E 36 and E 44 show that gelatine and caustic soda at the strengths used had no fungicidal action of themselves.

To test further the influence of the acidity or alkalinity of the spreader upon the fungicidal action of colloidal sulphur some experiments were made in which the spreader Agral I was used. A preliminary trial was made, Exps. E 47 and E 48, to ascertain whether Agral I itself could exercise any fungicidal action, but it was found not to do so. The object of the next experiments was to compare soft soap and Agral I as spreaders with colloidal sulphur.

Exp. E 69*. 0·2 per cent. colloidal sulphur plus 0·5 per cent. soft soap.

Exp. E 70*. 0·2 per cent. colloidal sulphur plus 0·5 per cent. Agral I.

Eleven leaves were sprayed. In each experiment the mixture proved to be almost but not quite fungicidal, most of the patches being killed while a very few patches on some leaves developed a small number of isolated, clustered conidiophores at their edges.

The behaviour of colloidal sulphur having thus been shown to be the same with Agral I as with soft soap, the next step was to confer acidity upon the neutral spreader and see whether in the acid medium the fungicidal action of the colloidal sulphur was inhibited.

Exp. E 67*. 1 per cent. colloidal sulphur plus 0·5 per cent. Agral I plus 0·12 per cent. sulphuric acid.

Exp. E 68*. 0·5 per cent. Agral I plus 0·12 per cent. sulphuric acid.

Ten leaves were sprayed. In Exp. E 67 fungicidal action was obtained on all the leaves; on three of the leaves injury was caused, in the form of prominent brown spots consisting of dead cells, while no injury was apparent on the remaining leaves. In Exp. E 68 the action was non-fungicidal and more or less marked injury was caused to all the leaves.

SUMMARY.

1. The action of various forms of sulphur—ground sulphur, flowers of sulphur and colloidal sulphur—as fungicides against the hop powdery mildew (*Sphaerotheca Humuli*) was tested in a number of experiments.

When ground sulphur or flowers of sulphur was wetted by a soft soap solution and applied to the mildew patches in the form of a spray, the fungicidal action was always very marked and, under the conditions of the experiments, became complete with suspensions containing 5 gm. sulphur per 100 c.c. of 0·5 per cent. soft soap solution. Owing, however, to the settling of the sulphur particles during the process of spraying, the amount of sulphur actually applied to the fungus was not strictly in accordance with the strength of the suspension.

2. Striking differences in fungicidal action were obtained when gelatine or saponin were used in place of soft soap as a spreader. Spreaders of an acid nature, viz. gelatine and saponin, inhibited the fungicidal action. With alkaline spreaders other than soft soap, *e.g.* lime casein and soda casein and the dry-mix sulphur lime, the alkaline reaction favoured the fungicidal action of the sulphur.

3. Trials of a commercial colloidal sulphur (Ialine) showed that, except in series C (see p. 27), this preparation at the rate of 0·3 gm. per 100 c.c. of 0·5 per cent. soft soap solution was completely fungicidal.

4. The influence of the spreader upon the action of the colloidal sulphur was, as in the case of the wetted ground sulphur, clearly shown and proved to be of the same nature.

5. The possible influence of the spreader upon the hydrolysis of sulphur is discussed.

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(*Received August 22nd*, 1929.)

THE HYDROLYSIS OF SULPHUR IN RELATION TO ITS FUNGICIDAL ACTIVITY.

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The mechanism of the fungicidal action of sulphur "at a distance"—when the sulphur is applied, not direct to the fungus but to a heated surface—has already been investigated in this laboratory (8, 9). It was found that the volatile agent concerned in this action is gaseous sulphur and it was shown that, although sulphur vapour at ordinary temperatures is extremely toxic to the black currant gall mite, it has little definite and immediate effect upon the powdery mildews of couch-grass and of hop. Nor could evidence be obtained that the sulphur recondensed from the vapour in a finely divided form—"particulate" sulphur—possesses fungicidal properties markedly superior to those of ordinary sulphurs. It was therefore concluded that the initial stage of the fungicidal action of sulphur at a distance is the recondensation of the volatilised sulphur upon the fungus, where its action is essentially the same as that of sulphur applied direct to the leaf. The problem of the action of sulphur at a distance is thus reduced to that of the mechanism of the toxic action of sulphur in direct contact with the fungus.

It seems inevitable for the explanation of the toxic action of sulphur, that the formation of a sulphur derivative capable of solution or penetration into the fungal hyphae should be hypothecated. The simpler hypotheses, that gaseous sulphur is itself the active agent or that the recondensed sulphur is directly toxic by virtue of its particulate state, do not receive support from the previous work referred to above. On the other hand, these investigations, though not affording proof, confirmed strongly the view that actual contact with the sulphur particle is necessary before the fungus is killed. For this reason and because of the extreme slowness with which dusting sulphur exerts its toxic action—and it will be shown below that there are grounds for believing the former not merely the corollary of the latter—it seems probable that sulphur acts upon the Erysiphaceae through the formation of an active derivative. Previous investigators have carried out much work from this point of view and a summary of the various hypotheses so far advanced has been given in a previous paper (8).

The older explanations may be divided into those suggesting an oxidation of the sulphur to sulphur dioxide, sulphuric acid or pentathionic acid; on the other hand, other workers have regarded hydrogen sulphide as the active fungicide, implying a reduction of the sulphur (17). That hydrogen sulphide is the active derivative is supported by more recent work of Barker (1) and of Marsh (13). The former obtained indications of the formation, from sulphur dusted upon certain leaves, of a compound giving the reactions of hydrogen sulphide, whilst Marsh has established the fact that hydrogen sulphide exerts a general and powerful toxic effect upon fungi. The hypothesis that pentathionic acid is the toxic factor produced by the natural oxidation of sulphur has been extended by Young and Williams (19) to dusting sulphurs. The conclusions of Roach and Glynne (15), who were concerned with the reactions of sulphur in the soil, are also of interest in connection with the toxic derivative of sulphur. They obtained evidence that the toxicity of sulphur towards *Synchytrium endobioticum* is due to the formation in the soil of free thiosulphuric acid or of some closely related sulphur acid.

In the work upon the action of sulphur at a distance metallic copper was used as a substitute for the fungus, the blackening of the copper being employed to detect volatile sulphur. It appears not impossible that the action of sulphur upon copper may have some relationship to its action upon the mildew. The tarnishing of certain metals by sulphur vapour was studied by Chavestelon (4), who found that for the production of a stain at ordinary temperatures the presence of moisture was necessary. This result may be explained by the assumption that the action of sulphur upon the metal is due primarily to hydrolysis, a conclusion accepted by Bassett and Durrant (2). It was therefore decided to examine the idea that the action of sulphur upon the fungus is likewise primarily due to hydrolysis.

THE HYDROLYSIS OF SULPHUR.

At first sight the possibility that a substance so insoluble and so "hydrophobic" as ground sulphur can react with water appears remote. Yet the possibility supplies a sound explanation of the initial stages of the interaction of sulphur with solutions of alkali hydroxides. Moreover, the interaction of hydrogen sulphide and sulphurous acid yields sulphur and water. It is usual to represent the reaction in both cases by the summation equation:

$$3S + 3H_2O \rightleftharpoons 2H_2S + H_2SO_3 \qquad \ldots\ldots(1),$$

the formation of polysulphide and thiosulphate in the case of the alkaline hydrolysis being due to secondary reactions of sulphur with sulphide and sulphite.

That the hydrolysis of sulphur does not follow so simple a course is shown, however, by the intricate chemistry of Wackenroder's solution. Bassett and Durrant (2) have been able to explain many of the difficulties of this problem by the assumption that the first step in the interaction of hydrogen sulphide with sulphurous acid may be represented:

$$H_2S + H_2S_2O_5 \rightleftharpoons S(OH)_2 + HO.S.O.S.OH,$$

the sulphide reacting with pyrosulphurous acid to yield sulphoxylic acid and its pyro-derivative (which in turn would be hydrolysed to form sulphoxylic acid). Free sulphur is then formed by the interaction of further hydrogen sulphide with sulphoxylic acid, and the initial stage of the hydrolysis of sulphur would therefore be represented by the equation:

$$2S + 2H_2O \rightleftharpoons H_2S + S(OH)_2 \qquad \text{.....(2).}$$

Little fundamental work appears to have been carried out upon the mechanism of the alkaline hydrolysis of sulphur, but it is of interest to note that, as long ago as 1905, Pomeranz (14) suggested that the primary reaction was the formation of the sulphide and "hyposulphite"[1]:

$$2S + 4NaOH = Na_2S + Na_2SO_2 + 2H_2O$$
$$(2S + 2Na_2O \rightleftharpoons Na_2S + S(ONa)_2).$$

Now if either equation (1) or (2) be accepted as representing the mechanism of the hydrolysis of sulphur, it is evident that the reaction from left to right corresponds to a marked increase in hydrogen-ion concentration. Addition of hydroxyl ions will, in accordance with the principle of Le Chatelier, favour the reaction in this direction. Hydrolysis of sulphur will therefore be accelerated in alkaline solution; the ease with which the reaction between sulphur and solutions of alkali hydroxides proceeds supports this conclusion. It follows that, if the action of sulphur upon the Erysiphaceae be dependent upon the occurrence of this hydrolysis, the presence of alkali should enhance its potency as a fungicide.

It was from this standpoint that certain spray trials, recorded in the paper immediately preceding the present communication, were carried out. Owing to the short period of the year during which the biological material is available, it was found necessary to confine the

[1] The constitution of sodium hydrosulphite (hyposulphite) was, at that time, unknown and the formula given to it by Pomeranz corresponds to that of sodium sulphoxylate.

trials solely to an examination of this conclusion. It is hoped that an opportunity will arise for a critical survey of the fungicidal properties of the possible sulphur compounds arising from hydrolysis. Further, it is well to point out at the outset that the hypothesis has been tested only upon one fungus—the powdery mildew of the hop.

EXPERIMENTAL.

As full details of the technique and results of the spray trials have already been given it is only necessary to indicate the reasons which prompted the various experiments and to discuss the results from the aspect of the hydrolysis hypothesis.

For the application of dusting sulphurs in the form of a spray, the presence of a capillary active substance is necessary to enable a wetting of the sulphur particle. By the same means it is possible to bring the spray into intimate contact with the fungus, for unless a spreading agent be added, the spray collects into droplets and is prevented from uniformly wetting the fungus (3). The spreaders most frequently used in spraying practice include, on the one hand, soap and solutions of casein in sodium or calcium hydroxide solutions; on the other hand, solutions of gelatine or saponin. The soap solutions are, because of hydrolysis, alkaline in reaction whereas freshly prepared gelatine and saponin solutions, because of decomposition and possibly hydrolytic changes, rapidly become acid. To test the influence of acid and alkali media upon the fungicidal activity of sulphur therefore presents no chemical difficulty.

A serious difficulty was, however, encountered in the use of ground sulphur, namely, that means of determining the amount of sulphur applied and of ensuring an even distribution of the sulphur were not available. The suspensions were applied by means of a hand atomiser, but in spite of vigorous shaking much of the sulphur separated to the bottom of the container. Moreover, an estimate of the amount of sulphur applied gave no reliable figure, for the densely packed conidiophores acted as filters and the sulphur was concentrated upon the mildew patches. Determinations of the amount per unit leaf area of sulphur remaining upon the leaf at the end of the experiment gave untrustworthy results, perhaps because of loss of sulphur by mechanical means during the examination of the effects of the spray upon the fungus. The biological results secured upon different leaves in the same experiment were, however, consistent and it is extremely improbable that the differences in fungicidal action observed were due to different concentrations of sulphur upon the fungus.

To avoid this difficulty use was made, in certain experiments, of a commercial product—"Ialine" colloidal sulphur. This consists of a suspension containing approximately 50 per cent. elementary sulphur in an extremely finely divided form. It was chosen from a number of so-called "colloidal" sulphurs because of its apparent purity. The material was but slightly acid in reaction, no organic "stabiliser" could be detected and the only impurity found was a trace of sulphur in the form of sulphate. The application of sulphur in this form served to confirm the results secured with the wetted ground sulphur.

The influence of the spreader upon the fungicidal activity of sulphur.

Table I.

Exp. no.	Type of sulphur	Strength gm. per 100 c.c.	Spreader	Strength %	Gm. "free" sulphur per 100 c.c.	Effect on fungus
C 34	Ground	5·0	Soft soap	0·5	—	Fungicidal
C 32	„	5·0	Gelatine	0·5	—	Non-fungicidal
E 5	Ialine	2·0	Soft soap	0·5	0·991	Fungicidal
E 6	„	2·0	Gelatine	0·5	0·991	Non-fungicidal
E 13	Ialine	4·0	Soft soap	0·5	1·91	Fungicidal
E 14	„	4·0	Gelatine	0·5	1·91	Non-fungicidal to not quite fungicidal
C 50	Ground	5·0	Saponin	0·5	—	Non-fungicidal
C 49	„	5·0	Lime casein	0·136 gm. CaO per 100 c.c.	—	Non-fungicidal, perhaps fewer conidiophores than in C 50
C 57	Dry-mix sulphur lime	7·8	—	—	5·0	Fungicidal
C 58	Ground	5·0	Soda casein	1·0 casein 0·2 NaOH	—	Not quite fungicidal
C 71	Ground	5·0	Soda casein	0·5 casein 0·2 NaOH	—	Fungicidal or (rarely) almost fungicidal
E 38	Ialine	1·0	Saponin	0·5	0·515	Non-fungicidal
E 37	„	1·0	Soft soap	0·5	0·515	Fungicidal
C 53	Ground (washed)	5·0	Gelatine	0·5	—	Non-fungicidal
C 54	Ground	5·0	„	0·5	—	„

For the purpose of examining the influence of the spreader upon the fungicidal activity of sulphur, suspensions were prepared containing 5 gm. per 100 c.c. ground sulphur (Fortress brand). In Exp. C 34, the combined wetter-spreader employed was soft soap (0·5 per cent. solution), whilst for the opposite leaves at the same node (Exp. C 32) a 0·5 per cent. solution of gelatine was used with the sulphur. By this means a comparison of the behaviour of the two spray-fluids under conditions strictly similar both biologically and externally was possible. Such comparative trials are bracketed in the table. The soft soap, a commercial sample, contained neither free alkali nor resin; the gelatine

was Coignet No. 1, Fine, the solution being prepared immediately before use. The sulphur was mixed to a consistent paste with a small amount of the spreader solution, the remainder added and the whole shaken for 7 hours in an end-over-end shaker to break up the small agglomerates of sulphur. Sulphur wetted with soft soap solution proved fungicidal, that wetted with gelatine solution had little effect upon the fungus. Confirming experiments were carried out with the colloidal sulphur. It was known from previous work that the minimum strength required for complete fungicidal action was a 0·2 to 0·4 per cent. suspension containing 0·5 per cent. soft soap. Yet in Exps. E 6 and E 14, suspensions of colloidal sulphur at ten times these strengths did not attain fungicidal action when applied with gelatine.

Similar experiments were carried out using, as the alkaline spreader, various solutions of casein and, for the acid spreader, saponin. Ordinary commercial saponin (at 0·5 per cent.) was employed, while the casein was a commercial "light yellow" product. In Exp. C 50, saponin is shown to resemble gelatine in its ability to inhibit the fungicidal action of sulphur. In Exp. C 49 the lime casein solution was prepared by dissolving casein in a boiling solution of calcium hydroxide, the solution being filtered when cold. The removal of the small amount of free calcium hydroxide by carbonation may perhaps account for the ineffectiveness of the sulphur in this experiment. Suspensions of sulphur in a lime casein containing larger amounts of free calcium hydroxide were therefore employed, the particular spray used being the "Dry-Mix Sulphur Lime" recommended by Farley(7). This form of "wettable" sulphur, which enjoys considerable popularity in America, is prepared by intimately mixing 16 parts of dusting sulphur, 8 parts of hydrated lime and 1 part of casein spreader (for the experiments here described, a commercial lime casein "Kayso" was used). The mixture is applied at the rate of 25 lb. per 100 U.S. gallons, which approximates to 30 lb. per 100 Imperial gallons. For the purpose of comparison, however, a suspension of dry-mix sulphur lime containing 5 gm. sulphur per 100 c.c. was employed in Exp. C 57. The contrasting spray was prepared by dissolving casein in a dilute solution of sodium hydroxide, the proportions used being 1 gm. casein and 0·2 gm. sodium hydroxide per 100 c.c.

In the presence of the larger excess of free calcium hydroxide it will be seen that sulphur is again effective as a fungicide. On the other hand, in the presence of the soda casein spreader, the extent of fungicidal action was less. An attempt was made to enhance the toxic property of this

spray by employing less casein, whereby the excess of free sodium hydroxide was increased (Exp. C 71).

The inhibition of the fungicidal action of sulphur by solutions of saponin was confirmed by experiments with the colloidal sulphur (Exp. E 38), the fungus re-growing rapidly after spraying, whereas in the control experiment (Exp. E 37) the colloidal sulphur-soft soap suspension proved fungicidal.

It is well established that, under certain conditions, samples of ground sulphur, and especially of flowers of sulphur, acquire a definite acidity, arising perhaps by oxidation of the products of hydrolysis. An alternative explanation of the action of alkaline and acid spreaders in promoting and inhibiting the fungicidal action of sulphur might be that the removal of the acid coating of the sulphur particle is necessary before fungicidal action can occur. To test this possibility an experiment was carried out with sulphur washed with alkali prior to application. The ground sulphur was first wetted with an alcoholic solution of sodium hydroxide, filtered, and well washed with alcohol. As it was intended to apply the sulphur with gelatine solution, it became necessary to remove the alcohol to prevent precipitation of the gelatine. For this purpose use was made of Agral I (a wetting agent employed in textile processing); this spreader, when the washings were free from alcohol, was itself displaced by gelatine solution. The washed sulphur, after 4 hours' shaking, was immediately applied to the fungus (Exp. C 53). Treatment of the sulphur with alkali prior to application has therefore failed to prevent the inhibition of its toxic action by the "acid" spreader.

From the hypothesis that pentathionic acid is the toxic factor of sulphur and because of the instability of the alkali pentathionates, Young and Williams (19) deduced that the presence of basic materials would inhibit the toxicity of sulphur. This conclusion is not supported by the experiments described above.

The influence of the addition of alkali to the sulphur-"acid" spreader spray.

If the inhibition of the fungicidal action of sulphur by solutions of saponin and gelatine is due to the acidity of their solutions, it should be possible to restore the toxic action by a suitable addition of alkali. Accordingly a series of experiments were carried out, employing as the wetter-spreader solutions of gelatine and saponin to which small amounts of sodium hydroxide were added. The results obtained are summarised in Table II. Within three hours of preparation, the suspension employed

in Exp. E 29 was definitely acid in reaction and it was evident that insufficient alkali had been added to neutralise the acid products of decomposition of the gelatine. Larger amounts of alkali were therefore used in Exps. E 35 and E 43 and, in view of a possible fungicidal action of the free alkali, the control leaves were sprayed with the alkaline spreader alone.

Table II

Exp. no.	Type of sulphur	Strength gm. per 100 c.c.	Spreader	Strength %	Alkali added % NaOH	Gm. "free" sulphur per 100 c.c.	Effect on fungus
E 28	Ialine	2·0	Gelatine	0·5	Nil	1·03	Non-fungicidal
E 29	,,	2·0	,,	0·5	0·024	1·03	,,
E 35	Ialine	2·0	Gelatine	0·5	0·15	1·03	Fungicidal or almost fungicidal
E 36	Nil	—	,,	0·5	0·15	—	Non-fungicidal
E 43	Ialine	2·0	Gelatine	0·5	0·20	1·03	Almost fungicidal to not quite fungicidal
E 44	Nil	—	,,	0·5	0·20	—	Non-fungicidal
C 45	Ground	5·0	Saponin	0·5	0·20	—	Not quite fungicidal
C 46	Nil	—	,,	0·5	0·20	—	Non-fungicidal

Similar trials were carried out with the ground sulphur and in this case saponin was used as the "acid" spreader. It will be seen that the addition of alkali has greatly enhanced the fungicidal properties of the sulphur-gelatine and sulphur-saponin sprays and the experiments confirm the deduction that the inhibition of the lethal action of sulphur by these spreaders is due to the acidity of their solutions.

The influence of the addition of acid to the sulphur-"alkaline" spreader spray.

The experimental verification of the corollary that the addition of acid should reduce the fungicidal efficiency of the sulphur-soft soap and sulphur-alkali casein sprays is rendered impossible because of the interaction of the acid with the soap or alkali caseinate and the destruction of the spreading properties of the solutions. In this connection, however, use was again made of Agral I, for it was found that aqueous solutions of this sulphonated aromatic derivative remained approximately neutral for considerable lengths of time and that the addition of small amounts of acid was without apparent effect upon their spreading properties. Moreover, spray trials (summarised in Table III) revealed the fact that, in the presence of this wetter, sulphur is extremely effective against the mildew.

An interpretation of the results of the trials involving an addition of sulphuric acid is complicated by spray injury. It will be seen that

in Exps. E 63 and E 64 the fungus was killed upon the control leaves. Strictly these experiments should therefore be discarded, but as the fungus thrived upon neighbouring unsprayed leaves it is permissible to assume that the fungicidal action was real and due to the acidified

Table III.

Exp. no.	Type of sulphur	Strength gm. per 100 c.c.	Spreader	Strength %	Acid added % H_2SO_4	Gm. "free" sulphur per 100 c.c.	Effect on fungus
E 47	Ialine	1·0	Agral I	0·5	Nil	0·516	Fungicidal
E 48	Nil	—	„	0·5	Nil	—	Non-fungicidal
E 69	Ialine	0·2	Agral I	0·5	Nil	0·105	Fungicidal to almost fungicidal
E 70	„	0·2	Soft soap	0·5	Nil	0·105	„ „
E 67	Ialine	1·0	Agral I	0·5	0·12	0·516	Fungicidal (injury)
E 68	Nil	—	„	0·5	0·12	—	Non-fungicidal (injury)
E 63	Ialine	1·0	Agral I	0·5	0·245	0·516	Fungicidal (injury)
E 64	Nil	—	„	0·5	0·245	—	„

Agral I solution. In Exp. E 68 a re-growth of conidiophores upon portions of the leaf uninjured by the spray was observed whereas upon the sulphured leaves the fungus was killed. To what extent this action was indirect and resulted from leaf injury was indeterminable, but the experiment indicates that the fungicidal action of the sulphur-Agral I spray was not destroyed by the addition of acid.

It will be seen in Exps. E 47 and E 48, and E 69 and E 70, that Agral I is as efficient as soft soap in promoting the fungicidal action of the colloidal sulphur. This effectiveness is not referable to the alkalinity of solutions of Agral I, and it must be assumed that some other factor is involved in the promotion of the hydrolysis of sulphur in its presence. From this point of view, the above experiments cannot be said to fulfil satisfactorily their original purpose, but they are recorded in connection with the parallelism of the action of sulphur upon the mildew and upon copper.

The action of sulphur upon copper.

It was the possibility that the action of sulphur upon the mildew might bear some relationship to its action upon copper which gave rise initially to the idea that the hydrolysis of sulphur might explain its fungicidal action. Although it would be unwise to push the analogy too far, it is of interest to determine whether the presence of the various spreaders has an influence upon the production of the tarnish upon copper. A series of trials were therefore carried out in which strips of copper foil (cleansed by a short immersion in 1 per cent. potassium

cyanide solution) were placed in suspensions of sulphur in the various spreading agents employed in the spray trials. A 0·4 per cent. suspension of Ialine colloidal sulphur was used, and in Table IV the appearance of the copper foil after 1 hour's immersion at ordinary temperatures is recorded.

Table IV.

Sulphur suspended in	Stain produced
Water alone	Slight
0·5 % soft soap	Black
„ soda casein	Well developed
„ gelatine	Just apparent
„ saponin	Just apparent
„ Agral I	Well developed
„ „ plus 0·5 % tartaric acid	Black
„ „ plus 0·12 % sulphuric acid	Well developed
„ „ plus 0·24 % sulphuric acid	Well developed

The parallelism is well maintained. The inhibition of stain production by the saponin and gelatine solutions is noteworthy and the failure of the addition of acid to Agral I to influence the action of sulphur upon the copper is comparable with the results of the spray trials.

THEORETICAL CONSIDERATIONS.

Although perhaps premature, in that little experimental verification has yet been possible, it is profitable to discuss briefly the results secured by other workers in this field upon the basis of the suggestion that the fungicidal properties of sulphur are dependent upon its hydrolysis. An excuse for the theoretical nature of such an examination is that the hydrolysis hypothesis at least serves for the co-ordination of existing theories relative to the fungicidal action of sulphur.

For example, Goodwin and Salmon (10) observed that sulphur wetted with a solution of soft soap is a far more efficient and rapid fungicide (against the hop powdery mildew) than sulphur applied in powder form. This difference of action they attributed to the better and more frequent contact of fungus and sulphur particle secured in the case of the spray-fluid. An additional chemical explanation is provided by the promotion of the hydrolysis of the sulphur by the hydroxyl ion produced by the hydrolysis of the soap.

The importance of contact between sulphur particle and fungus has repeatedly been stressed (16) and light is thrown upon this action by the experiments conducted with copper foil. If clean copper foil be placed, at an angle inclined to the vertical, in a suspension of ground sulphur

or of Ialine colloidal sulphur in the presence of an alkaline spreader, the tarnishing of the copper is far more pronounced upon the upper side. It is upon this side that the sulphur particles will fall and it is probable that the increased stain is produced by the promotion of hydrolysis by the removal, as copper sulphide, of the hydrogen sulphide formed. It is not impossible that, in the case of the fungus, direct contact with the sulphur particle may bring about a more rapid removal of the products of hydrolysis whereby hydrolysis is accelerated and the fungicidal activity of the sulphur enhanced.

A consideration of the possible products of hydrolysis and their bearing upon existing hypotheses of the fungicidal action of sulphur reveals further interesting correlations. According to present-day views, the primary products of the interaction of sulphur and water are hydrogen sulphide and sulphoxylic acid.

The formation of hydrogen sulphide from sulphur is well shown by the colour changes (red, deepening to black), at ordinary temperatures, of the sulphur particle when shaken with dilute solutions of sodium plumbite. The experiments of Barker(1) and of Marsh(13) upon the formation of a compound giving the reactions of hydrogen sulphide from sulphur dusted upon certain leaves and fungi "sensitive towards sulphur" have already been mentioned. A possible explanation of the mechanism of this action is that the hydrolysis of the sulphur is accelerated either by an alkaline secretion from the leaf or fungus or by a specific reaction causing the removal of a product of hydrolysis. It may be noted in passing that attempts to produce a stain on lead acetate paper clamped to sulphur-dusted patches of the hop powdery mildew failed. That hydrogen sulphide possesses general fungicidal properties was shown by Marsh.

The formation of sulphoxylic acid by the hydrolysis of sulphur is not so easily demonstrated, but indications of its production are afforded by the blackening of the sulphur particle on shaking sulphur with ammoniacal solutions of silver nitrate or of mercuric cyanide. Owing perhaps to the fact that ammonium hydroxide solutions are but feebly basic, the production of sulphide reactions occurs with extreme slowness when sulphur is shaken with aqueous solutions of ammonia in the cold, and even in ammoniacal copper sulphate solutions the sulphur particle remains unblackened. With ammoniacal silver nitrate solutions, however, the sulphur particle is immediately turned brown, the tint rapidly deepening to black. This reaction was regarded by Williams and Young(18) as proof of the formation of pentathionic acid from sulphur

on exposure to air; but the precipitation of metallic silver from ammoniacal solutions of silver nitrate by derivatives of sulphoxylic acid, such as sodium hydrosulphite, is employed for the quantitative analysis of such compounds (11). The possibility that a similar reduction to the metal may occur when ammoniacal solutions of silver nitrate are shaken with sulphur is discarded by Williams and Young, firstly, because analysis of the precipitate formed shows it to be silver sulphide and, secondly, because no reduction to the metal occurs when sulphur is shaken with ammoniacal solutions of platinic chloride. Subsequent combination of the silver with sulphur is, however, to be expected, whilst the addition of sodium hydrosulphite to ammoniacal platinic chloride solution brings about no precipitation of the metal.

Although in the cases of *Synchytrium endobioticum* and the hop powdery mildew the conditions under which sulphur exerts its fungicidal action are vastly different, it is of interest to note that towards the former fungus Roach and Glynne (15) found thiosulphuric acid an active fungicidal sulphur derivative. The close chemical relationship between thiosulphuric and sulphoxylic acids was established by Bassett and Durrant (2).

Finally, the fungicidal properties of polysulphide sulphur, an ultimate product of the alkaline hydrolysis of sulphur, towards the powdery mildews are well established (5, 6, 12).

SUMMARY.

1. It is suggested that the fungicidal action of sulphur upon the Erysiphaceae is due primarily to hydrolysis.

2. The presence of alkali, which brings about an acceleration of this hydrolysis, should enhance the fungicidal properties of sulphur.

3. An explanation is thus afforded of the inhibition of the fungicidal action of sulphur against the hop powdery mildew (*Sphaerotheca humuli*) by gelatine or saponin solutions when employed as the spreader and of the promotion of fungicidal action in the presence of soft soap and alkali casein solutions.

4. Attention is drawn to analogies between the action of sulphur upon the mildew and upon copper.

5. Current views relative to the fungicidal action of sulphur are discussed from the standpoint of the hydrolysis hypothesis.

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(*Received August 22nd*, 1929.)

FACTORS AFFECTING THE YIELD AND QUALITY OF MILK.

II. VARIATIONS IN SUCCESSIVE LACTATIONS.

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The rate at which the production of dairy cows changes, from the time they come into the milking herd until they reach their maximum yearly yield, has been frequently studied. In the majority of cases the records available have been classified according to the age of the cows at the start of the records, and then the age of maximum production and the changes in production at various ages determined. On the other hand, in some pieces of work the ages of the cows have not been given, but the records have simply been taken in series and the rate of change through successive lactations studied. An effort will be made here to compare these two methods.

I. HISTORICAL.

In the first paper of this series Kay and M'Candlish (9) reviewed the reports in which the records were classified according to the ages of the animals, so it will only be necessary to summarise here those reports

in which the lactations were taken in direct succession rather than according to the ages of the animals.

A. Influence on milk yield.

In all reports it is generally agreed that the milk production increases during the first few lactations and then decreases, though there is no exact agreement as to the lactation which is most generally associated with maximum production. According to Kent (10) maximum milk yield is reached in the fourth lactation, and in the fifth according to Spann (17), while Wilson (18) and Mackintosh (12) state that this may occur in either the fourth or fifth lactation. Then Hammond and Sanders (8) conclude that it may be in the fifth or sixth lactation, while Gavin (6, 7), Beam (2) and Sanders (16) give the sixth lactation as the stage of greatest yield.

It was found by Bartlett (1) and Langmack (11) that the highest yields of milk were obtained in the sixth and seventh lactations, while Maynard and Myers (13), who attempted to eliminate the variations due to the weeding out of the cows, found the maximum to be in the eighth lactation, though only 14 out of the 79 cows available had completed eight lactations. Considerable disagreement in these reports is evident, but the majority would seem to put maximum milk production at about the fifth or sixth lactation.

B. Influence on fat yield.

Only two reports are available on this point, and while Langmack (11) states that the maximum fat yield occurs in the sixth or seventh lactation, Maynard and Myers (13) found it to be in the eighth lactation.

C. Influence on fat percentage.

There is greater agreement as to the influence of the lactation on the percentage of fat in the milk than as to its influence on the yield of milk and butterfat. It was found by Carlyle and Woll (3), Spann (17), and Monroe (14), that the fat tended to be high in the first lactation and then decreased, while Mackintosh (12) states that the fat percentage is highest in the first and second lactations and afterwards shows a slight decrease with age.

According to Eckles and Palmer (5) the variations in the average fat percentage among the first few lactation periods are not large enough to be of any practical significance, but the gradual decline in average test accumulates to a figure of considerable importance as the number of lactation periods increases. They found that among Jerseys the highest

fat percentage occurred during the first three lactation periods, but the chances were that it would be in the second and third. Holstein-Friesians almost invariably had the highest percentage of fat in the first lactation period, while among Ayrshires the highest fat content was found more frequently in the first than in subsequent lactations, though less frequently so than with the Holstein-Friesians.

On the whole the bulk of the evidence appears to show that the highest fat percentage is generally found in the first lactation, though in some cases there may be a rise to the second, or more exceptionally to the third lactation, with a subsequent fall.

II. THE DATA.

In a study of the influence of age on milk and butterfat production by Kay and M'Candlish (9) records of pure-bred Ayrshire cows collected by the Scottish Milk Records Association in the three south-western counties of Scotland—Dumfries, Kirkcudbright and Wigtown—were taken. The data used were for cows that had five or more consecutive lactations and the records were classified according to the age of the cow at the time of calving. If a cow was eligible in other ways she was included if she missed a record in one age year, or had two records in one age year. This was necessary as it is very seldom that there is exactly one calendar year between successive calvings. Ultimately there were available for study 738 cows with a total of 4380 lactations.

For the present study the records of the same animals were used, but they were classified strictly on the succession of the lactations, irrespective of the ages at which these started. This means that the grouping varies somewhat from that in the previous study, and records of 683 animals with 4160 lactations are now available. These are grouped according to the number of successive lactations, 5 to 11, available and the numbers of animals and lactations in the various groups are given in Table I.

Table I. *Cows and lactations used.*

Group	No. of cows	No. of lactations	
		Per cow	Total
A	301	5	1505
B	178	6	1068
C	98	7	686
D	63	8	504
E	34	9	306
F	8	10	80
G	1	11	11
Total	683	—	4160

For convenience in reference the groups with 5 to 11 lactations are lettered consecutively from *A* to *G*. As is to be expected the numbers of animals in the groups with the largest numbers of lactations are smaller than the others.

III. RESULTS OBTAINED.

The average milk and butterfat yields and the average fat percentage for all groups in successive lactations are given in Tables II, III and IV.

Table II. *Average milk production, in pounds.*

Lactation / Group	1	2	3	4	5	6	7	8	9	10	11	Av.
A	6280	6373	6979	7168	7214	—	—	—	—	—	—	6803
B	6408	6562	7020	7431	7672	7551	—	—	—	—	—	7107
C	6740	6662	6913	7102	7631	7679	7259	—	—	—	—	7141
D	6774	7143	7100	7313	7516	7947	7973	7663	—	—	—	7429
E	6342	6933	7694	7500	7154	7335	7547	7951	7487	—	—	7327
F	6165	6925	7844	8695	8178	8305	7975	7911	7471	7454	—	7692
G	4900	6830	7290	8270	7900	8010	8440	8750	8640	9030	9770	7985
Av.	6424	6570	7038	7276	7430	7647	7561	7785	7511	7629	9770	7071

Table III. *Average fat production, in pounds.*

Lactation / Group	1	2	3	4	5	6	7	8	9	10	11	Av.
A	242·9	241·7	263·6	269·4	273·2	—	—	—	—	—	—	258·1
B	251·2	246·4	264·9	281·1	285·7	284·4	—	—	—	—	—	269·0
C	259·1	250·5	262·5	267·3	287·1	285·5	271·7	—	—	—	—	269·1
D	257·7	268·4	264·9	273·0	279·0	296·4	298·6	289·8	—	—	—	278·5
E	247·3	255·9	284·0	278·7	256·6	273·1	278·1	294·6	276·6	—	—	271·7
F	249·4	265·0	310·0	322·1	282·6	314·2	297·8	296·0	270·8	268·0	—	287·6
G	189·8	231·1	261·2	290·9	290·2	280·2	325·9	327·0	307·2	303·2	331·6	285·3
Av.	249·0	247·6	265·5	273·6	278·3	286·2	282·4	292·2	276·2	271·9	331·6	266·8

Table IV. *Average fat percentages.*

Lactation / Group	1	2	3	4	5	6	7	8	9	10	11	Av.
A	3·87	3·79	3·78	3·76	3·79	—	—	—	—	—	—	3·79
B	3·87	3·75	3·77	3·78	3·72	3·77	—	—	—	—	—	3·78
C	3·84	3·76	3·80	3·76	3·76	3·72	3·74	—	—	—	—	3·77
D	3·80	3·76	3·73	3·73	3·71	3·73	3·75	3·78	—	—	—	3·75
E	3·90	3·69	3·69	3·72	3·59	3·72	3·68	3·71	3·69	—	—	3·71
F	4·05	3·83	3·95	3·70	3·46	3·78	3·74	3·74	3·62	3·60	—	3·74
G	3·87	3·38	3·58	3·52	3·67	3·50	3·86	3·75	3·55	3·36	3·39	3·57
Av.	3·88	3·77	3·77	3·76	3·75	3·74	3·73	3·75	3·68	3·56	3·39	3·77

A. MILK YIELD.

In all groups the milk yield tends to rise through successive lactations for a time, and then, provided sufficient lactations are available, the average yield tends to fall. The first two groups show the highest

average yield in the fifth lactation, and the three succeeding groups in the sixth, seventh and eighth lactations. The group with 10 lactations shows the highest average in the fourth lactation, while the group with 11 lactations does not reach its highest production until the last lactation, but this may be neglected as there is only one animal in the group. Perhaps only the first four groups have sufficient animals to be of importance. When all records are combined it is found that the yield increases from the first to the sixth lactation.

B. FAT YIELD.

The fat yields show variations similar to those of the milk yields. The first three groups show the maximum in the fifth lactation, the next two in the seventh and eighth respectively, and group *F* in the sixth lactation, while the small remaining group does not reach a maximum until the last lactation. On the combination of all records the maximum is obtained in the sixth lactation.

It should also be noted that though there is a general rise from the first to the fifth or sixth lactation, there is in many cases a fall from the first to the second lactation with a subsequent rise, or where there is not a fall here there is only a relatively small increase. This is in accord with the results already obtained by Kay and M'Candlish (9).

C. FAT PERCENTAGE.

In all groups the fat percentage is highest in the first lactation, shows a marked drop in the second, then is fairly steady for a time with a downward tendency and in the advanced lactations shows a considerable drop.

IV. DISCUSSION OF RESULTS.

Now that the variations from lactation to lactation are available it will be necessary to discuss the significance of these and compare the results obtained with those secured where the records were classified on the age basis.

A. MOST PRODUCTIVE LACTATION.

In the groups with the largest numbers of animals and the smallest numbers of successive lactations the highest yields of milk and butterfat are as a rule obtained in the fifth lactation, while in the groups with fewer animals but larger numbers of successive lactations the highest average yield is obtained, with one exception, in some of the higher

lactations. On the average the sixth lactation is the most productive in both milk and butterfat, but the increase from the fifth lactation can be neglected as the groups with the largest number of cows show only a very slight increase from the fifth to the sixth lactation. For all practical purposes the milk and butterfat yields can be taken as increasing from the first to about the fifth lactation, being held fairly steady for a few subsequent lactations, and then declining.

The fat percentage is at its highest in the first lactation, drops considerably in the second, is fairly steady though with a downward trend for a few lactations, and then shows a tendency to fall markedly in the highest lactations.

B. Comparison of age and lactation.

In Table V the averages for all groups combined are given along with the averages for the various ages obtained by Kay and M'Candlish (9) in the first paper of this series. It can be seen that the results from the two studies are quite comparable. The difference in the average for all records included in each study is only 10 lb. of milk and 0·5 lb. of butterfat, while the average fat percentages are the same.

Table V. *Comparison of records averaged by ages and by lactations.*

According to age				According to lactation			
Age	Milk lb.	Fat lb.	Fat %	Lactation	Milk lb.	Fat lb.	Fat %
3	6407	248·2	3·87	1	6424	249·0	3·88
4	6619	248·7	3·76	2	6570	247·6	3·77
5	7049	265·9	3·77	3	7038	265·5	3·77
6	7257	271·6	3·74	4	7276	273·6	3·76
7	7439	279·3	3·75	5	7430	278·3	3·75
8	7514	282·8	3·76	6	7647	286·2	3·74
9	7722	288·2	3·73	7	7561	282·4	3·73
10	7429	279·5	3·76	8	7785	292·2	3·75
11	7703	285·9	3·71	9	7511	276·2	3·68
12	7899	255·2	3·23	10	7629	271·9	3·56
13	10095	348·1	3·45	11	9770	331·6	3·39
Average	7061	266·3	3·77	Average	7071	266·8	3·77

C. Should age or lactation be used in correcting records?

The correction most frequently applied to milk records in the past has been that for the age or maturity of the cow. This has been done in two ways, either strictly according to the age of the cow at the time of calving, or according to the number of the lactation. The first method has been the more popular though the latter has also been used to a considerable extent.

According to Sanders (15) it is usually impossible to obtain the exact ages of the animals in commercial herds in England, though the number of the lactation can be secured. Under the milk recording scheme in Scotland, on the other hand, the ages of all pure-bred animals, and of the great majority of others, can be secured.

Then in a later paper Sanders (16) states that it is not the number of lactations a cow has had, but the number of years she has lived which determines the variation in production. In opposition to this are Davydov and Fedotova (4) who are of the opinion that there is a closer correlation between milk production and the number of lactations, than between milk production and age.

From these facts it is evident therefore that a comparison of the two methods of correcting records for the maturity of the cow—according to age, and according to the lactation—is desirable.

It appears from Table V that the results obtained by the two methods of tabulation are very similar, and when the records for each age were expressed as a percentage of the record at 7 years of age, and the record for each lactation expressed as a percentage of the yield in the fifth lactation, the same results were found. It may be concluded therefore that when the age is known the correction can be made according to the age of the animal, but where the age is not available and the number of the lactation is known then the correction can be made according to the lactation, and comparable results will be obtained.

Correction factors have been worked out for successive lactations and these are given in Table VI along with the figures previously obtained for age proper. Here it is seen that the variations between the two sets of factors are never more than 1 per cent.

Table VI. *Correction factors.*

For age			For lactation		
Age	Milk	Fat	Lactation	Milk	Fat
3	1·16	1·13	1	1·16	1·12
4	1·12	1·12	2	1·13	1·12
5	1·06	1·05	3	1·06	1·05
6	1·03	1·03	4	1·02	1·02
7	1·00	1·00	5	1·00	1·00

In using these factors the record at any given age, or in any given lactation, when multiplied by the appropriate factor, gives the equivalent production at 7 years of age, or in the fifth lactation. Beyond this point no correction is necessary as the later variations are small and irregular.

V. SUMMARY.

1. Milk and butterfat production rise to about the sixth lactation, but beyond the fifth lactation the increase may be neglected.

2. The lactation of maximum production may be taken as the fifth which corresponds to 7 years of age.

3. Beyond the fifth lactation the milk and butterfat yields vary irregularly but show a downward tendency.

4. The fat yield shows either a fall or only a very small increase in the second lactation.

5. The fat percentage is highest in the first lactation, drops markedly in the second, remains fairly steady for a few lactations, though with a slight downward tendency, and then falls off in the higher lactations.

6. Results obtained by applying correction factors for either age or lactation are quite comparable and choice in the method to be used will depend largely on the original data available.

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(*Received August 28th*, 1929.)

NUTRITIVE VALUE OF PASTURE.

V. PASTURE GRASS CONSERVATION: THE INFLUENCE OF ARTIFICIAL DRYING ON THE DIGESTIBILITY OF PASTURE HERBAGE.

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Introduction.

Recent research at Cambridge (1, 2, 3, 4) has led to the following three conclusions: (1) Young leafy pasturage has a higher feeding value than has hitherto been suspected. Its dry matter has the character of a protein concentrate of high digestibility and nutritive value. (2) When pastures are closely grazed, either continuously or at regular intervals, this concentrated character is retained throughout the entire season. (3) Certain species of grasses, such as creeping bent, which under an inefficient system of grazing have only an indifferent feeding value, display, under conditions of "direct" or "rotational" close-grazing (4), a composition and a feeding value approximating to those of the more esteemed grasses such as perennial rye grass, cocksfoot and rough-stalked meadow grass.

The discovery of the protein-concentrated character of young pasture grass naturally suggested the idea of conserving the surplus produce of pastures and, where feasible, the cuttings from lawns and playing fields, for feeding to animals during winter as a substitute for oil cakes and other protein-rich foods. For this purpose, it was clear that two methods of preservation were possible, namely, artificial drying and ensilage. It was felt, however, that large-scale work on the drying of young grass should be put off until it had first of all been ascertained whether the temperature requisite for suitably quick drying had any adverse influence on the digestibility of the herbage.

The impression is generally entertained that the effect of heat on a moist feeding stuff is to depress its digestibility, in particular, the digestibility of its protein constituent. This view seems to have arisen from the work of early investigators on the effect of cooking such foods as meat (5), the conclusions being based on measurements, before and

after cooking, of the digestion coefficient of the protein by means of Stutzer's artificial digestion (pepsin-HCl) method. It is not substantiated, however, by the results of more recent investigations in which animals were used. For example, the writer has demonstrated that when maize is submitted to (*a*) cooking under kitchen conditions, (*b*) steaming followed by rolling into flakes, these processes give rise to a significant *improvement* of the digestibility of the protein of the grain (6). It has also been shown that the high temperature employed in the factory for drying wet sugar beet pulp does not result in any depression of the digestibility of the protein in the food (7).

It is the purpose of this communication to record the results of two separate enquiries into the influence of artificial drying on the digestibility of young leafy pasturage. It will be shown that such herbage can be dried down without impairing the high nutritive properties of the fresh grass. In the first trial, grass cut at weekly intervals was dried at the temperature of steam; in the second trial, cuttings from playing fields were dried by direct heat in a kiln.

I. Digestion trials on steam-dried grass.

During the season of 1927, Imperial Chemical Industries, Ltd., collaborating with the Cambridge School of Agriculture, undertook the cutting, at weekly intervals, of some acres of grassland in the vicinity of their factory at Billingham. Soon after cutting, the herbage was dried down in steam-heated troughs, the material being agitated mechanically during the process to facilitate the drying. The dried product was later compressed into cakes by means of hydraulic presses.

The dried grass cakes as made in this trial measured 6 in. by 5 in. by 1 in. and were of such a density that 40 cubic feet of the compressed material weighed 1 ton. A higher density than this, however, is attainable, since samples of dried grass briquettes made at a somewhat later date by Messrs Shirtliff of Letchworth were so dense that as little as 26 cubic feet weighed 1 ton. The dried grass cakes had retained the green colour of the fresh grass and possessed a pleasant, fragrant odour. When soaked in water, they swelled up considerably and disintegrated. They were consumed readily by sheep, bullocks and dairy cows both in the dry condition and also after soaking in water. When fed dry, the grass cakes were first broken up in a cake crusher suitably adjusted.

In Table I are recorded the results of the analysis of typical samples of the dried grass cakes. It will be noted that the data are such as have

been shown, in preceding communications, to be typical of pasture grass in its early stages of growth.

Table I. *Composition of typical samples of dried grass cakes (dry matter basis).*

	Sample 1 %	Sample 2 %
Crude protein	27·90	29·15
Ether extract	7·71	6·95
N-free extractives	38·84	33·33
Crude fibre	16·03	15·93
Ash	9·52	14·64
True protein	23·27	24·70
"Amides"	4·63	4·45
Lime (CaO)	1·48	1·27
Phosphoric acid (P_2O_5)	0·90	1·00
Silica	3·38	6·16

The percentages of moisture in the grass cakes ran from 8 to 9 per cent. It should be mentioned that some of the samples were characterised by an unusually high ash content, composed largely of silica. In one case, for example, the ash and silica comprised 21·5 and 13·5 per cent. of the cake respectively. This was obviously the result of the inclusion of soil in the herbage during cutting operations, such contamination being liable to occur when cutting *every week* during the dry, slow-growing part of the season. Worm-casts during the late part of the season may also give rise to soil contamination. Since carrying out this work, it has been shown that cutting may be done at intervals of a fortnight or three weeks instead of one week(3, 4); the risk of inclusion of soil may therefore be reduced by adopting a more lenient system of cutting. It will be advisable, however, that grass-drying appliances should be provided with devices for removing any included soil during the process of drying.

It is of interest to record that samples of the dried grass cakes have been kept for more than two years in an open box under laboratory conditions without displaying any deterioration whatsoever in respect of colour and smell. After this period of storage, their moisture content is still in the neighbourhood of 8 to 9 per cent.

The digestion trials of the dried grass cakes were carried out on two pure-bred Suffolk wether sheep which had already been employed for the purposes of the 1927 trials of fortnightly-cut grass. They were about two years old. The experimental period of collection and analysis of the excreta was of 14 days' duration. Each animal received a daily ration of 1400 gm. of the dried grass, which was fed, and eagerly consumed,

in the dry condition. The mean composition of the dried grass is shown in Table II.

Table II. *Mean composition of dried pasture grass used in digestion trials (dry matter basis)**.

	%		%
Crude protein	26·07	Lime (CaO)	0·97
Ether extract	6·43	Phosphoric acid (P_2O_5)	0·93
N-free extractives	39·45	Silica	4·59
Crude fibre	15·41	True protein	22·15
Ash	12·64	"Amides"	3·92

* Mean moisture content as fed 12·30 %.

Table III. *Digestibility of dried grass cakes.*

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
SHEEP V.							
Consumed daily	1228·00	1072·78	320·14	78·96	484·45	189·23	155·22
Voided daily	329·00	238·62	69·02	25·86	103·31	40·43	90·38
Digested daily	899·00	834·16	251·12	53·10	381·14	148·80	64·84
Digestion coefficients of dried grass, %	73·21	77·76	78·44	67·25	78·68	78·64	41·77
SHEEP VI.							
Consumed daily	1228·00	1072·78	320·14	78·96	484·45	189·23	155·22
Voided daily	321·60	230·78	70·78	20·52	97·80	41·68	90·82
Digested daily	906·40	842·00	249·36	58·44	386·65	147·55	64·40
Digestion coefficients of dried grass, %	73·81	78·49	77·89	74·01	79·81	77·97	41·49
Mean digestion coefficients, %	73·5	78·1	78·2*	70·6	79·3	78·3	41·6

* Protein digestion coefficient as determined artificially by pepsin-HCl = 81·0 %.

COMMENTS ON TABLE III.

Unfortunately it was not feasible to carry out digestion trials of the fresh, weekly-mown herbage from which the dried grass cakes were made, so that it is difficult to draw a direct conclusion respecting the effect of the temperature of drying on digestibility. A sufficiently reliable opinion may be obtained, however, by comparing the results with those obtained in 1925 and 1926 in a series of digestion trials of pasture herbage cut at weekly intervals throughout the season and fed in the fresh condition. The comparison is shown in Table IV.

It will be noted that the digestion coefficients of the dried grass cake (with the exception of that for the ether extract) are somewhat lower than the *best* figures for the herbage consumed in the fresh condition in the 1925 and 1926 trials. This does not necessarily imply, however, that

artificial drying leads even to such a slight depression of digestibility, since a difference of this magnitude might equally well be attributed to other causes, such as: (1) The use of different pairs of animals in the several trials. (2) The influence of meteorological factors on the digestibility of herbage cut at weekly intervals. It has been demonstrated (4) that grass cut at intervals of a week is much more liable to depression of digestibility during spells of dry weather than is the case with grass cut every fortnight or every three weeks. It is to be regretted, therefore, that the writers have no information which would enable them to decide how far meteorological factors should be taken into account in considering the results of the present trial.

Table IV. *Summary of digestion coefficients of dried grass cakes and of weekly-cut herbage fed in the fresh condition.*

	Dried grass cakes (present trial)	1925 season (1)			1926 season (2)			Mean for 1925 and 1926
		Min.	Max.	Mean	Min.	Max.	Mean	
	%	%	%	%	%	%	%	%
Organic matter	78·1	74·0	83·6	78·7	75·8	83·1	79·9	79·3
Crude protein	78·2	76·6	85·4	80·6	80·6	84·1	82·4	81·5
Ether extract	70·6	39·7	60·6	54·1	52·0	68·1	60·3	57·2
N-free extractives	79·3	75·0	88·1	80·5	75·6	85·4	80·1	80·3
Crude fibre	78·3	72·9	84·2	78·5	73·7	85·8	81·9	80·2

The fairest comparison, under the circumstances, is between the results for the dried grass cakes and the mean figures for the dual series of digestion trials carried out in 1925 and 1926. With the exception of the results for the ether extract, which display a difference markedly in favour of the dried grass cakes, these sets of results are sufficiently similar to warrant the conclusion that the process of drying young grass at 100° C. leads to no significant depression of digestibility. In particular, it may be noted that the high degree of digestibility which characterises the protein constituent of young leafy pasturage is retained after drying. This finding, which is of primary importance in view of the protein-rich character of young grass, is confirmed by a further series of experiments. During the carrying out of the 1925 investigation, the samples of weekly-cut herbage for analysis were dried on a hot plate, the surface of which registered 100° C. The drying process was completed to constant weight in a steam oven. From such dried material, the composite samples representative of the grass fed to the sheep over the periods of the several digestion trials were made up. As the digestibility of the protein in these composite samples was determined artificially by

Stutzer's pepsin-HCl method, it was possible to compare the results so obtained with those arrived at in the sheep digestion trials when the freshly-cut herbage was tested. The comparison is set out in Table V.

Table V. *Digestion coefficients of protein of young grass as determined on the freshly-cut herbage (in the animal) and on the dried herbage (by the action of pepsin-HCl).*

Period of experiment	1 %	2 %	3 %	4 %	5 %	6 %	7 %	8 %	9 %
Fresh grass (by animal experiment)	79·3	85·4	81·1	78·4	77·1	76·6	78·9	83·4	84·0
Dried grass (by action of pepsin-HCl)	83·5	85·1	81·7	82·4	80·1	78·5	81·3	85·1	84·5

It will be noted that the data in Table V are entirely consistent with the conclusion that young grass can be dried artificially at 100° C. without suffering any depression in respect of the digestibility of its protein constituent. Indeed, the digestion coefficients determined by the action of pepsin-HCl on the dried grass throughout run slightly higher than those obtained in the digestion trials of the freshly-cut grass. This behaviour, however, is perfectly normal. Assuming the process of drying to be without effect on the digestibility of the protein, it would be anticipated that the figures determined by the artificial method would be somewhat higher than those obtained in the animal trials, the discrepancy arising as a consequence of the presence of metabolic nitrogenous material in the faeces of the sheep.

The conclusion from the foregoing digestion trial to the effect that the high nutritive value of fresh pasturage is not lost as a result of artificial drying is borne out by the results of feeding trials which were carried out on the University Farm at Cambridge during the winter of 1927–8. These experiments have demonstrated that the dried grass cake is able to replace oil cake in the winter rations of dairy cows and fattening bullocks. As accounts of these feeding trials will appear separately in due course, it will only be necessary at this point to give the following brief statement compiled from information kindly furnished by the writers' colleagues, Mr W. S. Mansfield, M.A. and Mr F. H. Garner, M.A.

A number of steers of the Shorthorn type, with an average weight of $8\frac{1}{2}$ cwt. at the commencement of the trial, were fed in a covered yard on a ration composed of 10 lb. dried sugar beet pulp, 14 lb. silage, 5 lb. meadow hay and 5 lb. dried grass cake. Towards the end of the feeding period, the dried grass cake was increased to 7 lb. and the hay

reduced to 3 lb. The cattle did particularly well. They averaged a steady 2 lb. per day live-weight increase during the first two months of fattening and about $1\frac{3}{4}$ lb. per day for the last month. They displayed a beautiful "bloom" at the end of the experiment, and three local butchers, each of whom purchased two of the animals, would not believe that these cattle had not been receiving a generous ration of "cake," that is, cake in the accepted sense of the word. The quality of the beef was excellent, and one of the slaughtermen, enquiring as to the feeding of the cattle, remarked that from the appearance of the carcase, he would have supposed it was the best "grass meat."

Equally successful results were obtained in the use of the dried grass cake for dairy cows, when the whole of the normal cake allowance and minerals (steamed bone flour) in the rations of the animals was replaced, at the rate of 4 lb. per gallon of milk, by a mixture of 3 parts of dried grass cake and 1 part of crushed oats, the oats being included to balance the excess of digestible protein in the grass cake. In a further trial carried out during the winter of 1928–9, the allowance of the mixture of dried grass cake and crushed oats was reduced to $3\frac{1}{2}$ lb. for every gallon of milk without diminishing the milk-producing capacity of the experimental animals.

II. DIGESTION TRIALS ON KILN-DRIED GRASS.

When the proposals for conserving young grass by the process of artificial drying were first put forward, it was natural that many farmers should be anxious to utilise kilns and oasts for this purpose, since during the best period of the pasture season, such drying appliances are often standing idle. It was necessary at that early stage, however, to point out that such methods of drying might possibly have an adverse effect on the digestibility of the pasture herbage and to advise against starting the process on a large scale until the effect of kiln-drying on the nutritive value of the grass had been tested.

The writers have been able to carry out an investigation into this question as a result of facilities kindly granted by Capt. R. S. Hicks, on whose estate at Wilbraham (Cambs.) stands a two-storeyed brick kiln which was erected some years ago for the purpose of drying seed after electrical treatment. It would serve no useful purpose to describe the construction of the kiln in detail, since manifestly, in view of its original purpose, it was designed for securing slow drying at a moderate temperature rather than for quick drying at high temperatures. It will suffice to state that the floor of the drying chamber is formed of red tiles

perforated with tiny holes through which come the fumes from an anthracite fire.

The sample of dried grass which was used for the purpose of the digestion trials was obtained in the following manner. A 4 in. layer of grass cuttings, taken from a college playing field in Cambridge during May, 1929, was placed on the floor of the drying chamber, which at this stage registered 100° C. Steam began to come off copiously at once. The floor temperature during the drying process rose gradually to 115° C., but the temperature of the grass, so long as the latter still contained appreciable amounts of moisture, did not rise beyond 50° C. Only in the final stages, when the grass was almost dry, did its temperature rise beyond 90° C. At intervals of 30 minutes, the grass was turned by means of a fork, and after 3 hours of drying it was removed from the chamber. At this stage, its moisture content was 2·40 per cent. It had retained the green colour of the fresh grass and possessed quite a pleasant smell.

Table VI. *Mean composition of kiln-dried grass used in digestion trials (dry matter basis)**.

	%		%
Crude protein	23·60	Ash	12·70
Ether extract	6·39	Lime (CaO)	1·83
N-free extractives	41·01	Phosphoric acid (P_2O_5)	1·00
Crude fibre	16·30	Silica	4·30

* Mean moisture as fed 9·00 %.

Table VII. *Digestibility of kiln-dried grass.*

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Sheep IX.							
Consumed daily	910·00	794·43	214·76	58·15	373·19	148·33	115·57
Voided daily	227·10	162·60	46·65	15·03	74·35	26·57	64·50
Digested daily	682·90	631·83	168·11	43·12	298·84	121·76	51·07
Digestion coefficients of dried grass, %	75·04	79·53	78·28	74·15	80·08	82·09	44·19
Sheep X.							
Consumed daily	910·00	794·43	214·76	58·15	373·19	148·33	115·57
Voided daily	226·30	164·06	46·84	15·59	71·78	29·85	62·24
Digested daily	683·70	630·37	167·92	42·56	301·41	118·48	53·33
Digestion coefficients of dried grass, %	75·13	79·35	78·19	73·19	80·77	79·88	46·15
Mean digestion coefficients, %	75·1	79·4	78·2*	73·7	80·4	81·0	45·2

* Protein digestion coefficient as determined artificially by pepsin-HCl = 83·9 %.

Digestion trials were carried out on the dried grass obtained in this process, two pure-bred Suffolk wethers, aged about 16 months, being

employed for the purpose. Each sheep received 1000 gm. of the grass daily and the ration was given in the dry condition. The faeces of the sheep were absolutely normal throughout the period. The mean composition of the grass as fed to the experimental animals is shown in Table VI.

COMMENTS ON TABLE VII.

With a view to ascertaining the effect, if any, of kiln-drying on digestibility, the results of the present investigation are compared in Table VIII with the mean results obtained during 1925(1) and 1926(2) in a series of digestion trials of pasture grass cut at weekly intervals throughout the season and fed to the sheep in the fresh condition. Fuller details concerning the last-named trials are given in Table IV.

Table VIII. *Summary of digestion coefficients of kiln-dried grass and of weekly-cut herbage fed in the fresh condition.*

	Kiln-dried grass (present trial) %	Freshly-cut grass (mean of 1925 and 1926 trials) %
Organic matter	79·4	79·3
Crude protein	78·2	81·5
Ether extract	73·7	57·2
N-free extractives	80·4	80·3
Crude fibre	81·0	80·2

It is safe to conclude from the comparative data in Table VIII that the process of drying in the kiln had led to no significant depression of the digestibility of the herbage. The digestion coefficients of the organic matter, N-free extractives and crude fibre are remarkably similar in both cases. The digestion coefficient of the protein of the dried grass is slightly lower than that of the freshly-cut grass, but this small difference, if significant at all, appears to be related to the amount of metabolic nitrogenous material in the faeces of the sheep when subsisting on the diet of dried grass, rather than to an effect of drying, since the figure obtained for the digestibility of the protein of the dried grass by means of Stutzer's pepsin-HCl method, namely, 83·9 per cent., was appreciably higher than the animal figure. It will also be noted that the ether extract of the kiln-dried grass was much more digestible than the corresponding fraction of the freshly-cut grass, a peculiar finding which was also obtained in the digestion trial of steam-dried grass.

SUMMARY.

The recognition of the protein-concentrated and highly digestible character of young leafy pasturage has led to proposals for conserving such herbage by artificial drying for feeding to farm animals during winter as a substitute for oil cakes. Before contemplating the adoption of such proposals on a commercial scale, however, it was necessary to demonstrate that young pasturage could be dried artificially without losing its highly digestible nature.

That this is possible is clear from the results brought forward in this communication. It has been shown that young grass does not suffer any depression in respect of digestibility when it is dried (*a*) at the temperature of steam, or (*b*) by direct heat in a kiln.

ACKNOWLEDGMENTS.

The writers have pleasure in expressing their indebtedness to Imperial Chemical Industries, Ltd. and, in particular, to Sir Frederick Keeble, F.R.S., under whose direction the dried grass cake, used in the present investigation, was manufactured. They also wish to acknowledge the generous help of Capt. R. S. Hicks, who permitted his seed-drying kiln to be used for the drying down of grass for experimental purposes. Thanks are also due to the writers' colleagues, Messrs D. B. Norman, M.A. and M. H. French, B.A., for assistance in the different phases of the experiments.

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(*Received September 13th*, 1929.)

A NOTE ON THE VALUE OF UNIFORMITY TRIALS FOR SUBSEQUENT EXPERIMENTS.

By H. G. SANDERS, M.A., Ph.D.

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It is now generally accepted that the soil of any field may be assumed to be markedly variable, and that, in consequence, treatments or varieties must be replicated in experimental trials, so that the error caused by soil differences may be estimated, and the significance of the results appreciated: variations in fertility over the area covered are always found in uniformity trials—that is to say, trials in which the whole area was treated exactly similarly, but plots in which were harvested, and the produce weighed, separately. In view of these irregularities, one possibility of increased precision appears to lie in carrying out a preliminary uniformity trial, harvesting the crop separately in the various plots to be used for a subsequent experiment, and so mapping out the fertility of the field by the plot yields when all are treated alike: these figures could be used later to correct the yields of the plots when under experiment, and so to circumvent to a certain extent the field experimentalist's bugbear—soil heterogeneity: it is conceivable that in this way a degree of precision might be attained with the actual experiment which could only be reached otherwise by a greater number of replicates than could well be managed, and that consequently the extra labour involved in preliminary uniformity trials might be justified. Naturally the possibilities depend entirely on the constancy of the plots in their relative productivity in different years, under different climatic conditions, and, usually, under other crops: this question could readily be explored were figures available giving the results of uniformity trials carried out over a series of years, on the same fields, sub-divided into the same plots, but such data are rare, and those discussed here are recognised as inadequate to give anything approaching a final solution. It was thought, however, that a note might serve to direct attention to the problem, and might stimulate others, who have access to suitable and more abundant data, to investigate them with the same end in view: it was also felt that some workers might be glad of an example of how the results of a uniformity trial

might be used in subsequent experiments on the same plots, so that the method of working is described in some detail, at the risk of appearing obvious.

THE METHOD OF USING THE INFORMATION.

If the yield of any plot under a preliminary uniformity trial (or its mean yield under a plurality of trials) be denoted by x, and its yield in the actual subsequent experiment by y, then some method of correcting y for x must be selected. At first sight the simplest procedure would appear to be to take the difference between the two—the efficacy of any one experimental treatment being tested by the mean value of $y - x$ for the plots on which it was carried out: if x and y were expressed as percentages of the mean yield of all plots in their respective years, then a positive value of $y - x$ would indicate that that particular treatment had been beneficial relative to the other treatments employed, and vice versa. Statistically, however, this method may lead to a loss, rather than a gain, in precision, for if V_x be the variance of x, then

$$V_{(y-x)} = V_y + V_x - 2r_{xy}\sqrt{V_x V_y}.$$

Consequently if there is no correlation between the plot yields in the two years, this method would add to the variance of y that of x: with V_x and V_y approximately equal, no gain at all would be made unless r_{xy} exceeded + 0·5, whilst if it were negative there would be a serious loss.

A gain may, however, be effected by means of the regression function between the two variables: if $V_{y \cdot x}$ denote the variance of y corrected for x by means of this (or, as it is sometimes stated "with x held constant"), then

$$V_{y \cdot x} = V_y(1 - r^2_{xy}) = V_y - \frac{(\mathrm{Cov}_{xy})^2}{V_x},$$

where Cov_{xy} is the covariance between x and y, or the mean product of their deviations from their means. In this case there is a definite gain if x and y are at all closely related (positively or negatively)—that is, if the produce of an individual plot in one year is any guide to its performance in another. It will be realised that $V_{y \cdot x}$ gives the variance of y corrected by the regression equation $y = bx$, where b is equated to Cov_{xy}/V_x: accordingly it must not be used to test the significance of the difference between the actual mean plot yields given by any two treatments, but that between the mean values of $y - bx$ given by them: if n be the number of replicates, the figures compared will be $S\left(\frac{y - bx}{n}\right)$, or

$\frac{1}{n}(Sy - bSx)$: since, with a random distribution of the treatments over the plots, the values of Sx will tend to be constant, it follows that the differences between these figures will be of the same order of magnitude as the differences between the means of y, and consequently any reduction effected in calculating $V_{y \cdot x}$ from V_y will be a direct gain in precision. It can readily be seen that in all circumstances the variance of $y - bx$ will be less than that of $y - x$, for

$$V_{y \cdot x} < V_{(y-x)},$$

if $$V_y (1 - r^2_{xy}) < V_y + V_x - 2r \sqrt{V_x V_y},$$

if $$(\sqrt{V}_x - r \sqrt{V}_y)^2 > 0,$$

which is necessarily true, since a square cannot be negative.

THE DATA.

Uniformity trials were carried out between 1906 and 1911 on two fields at Aarslev (Denmark) (1), which provide (very limited) data to test the possibilities. One field (A_2) was divided into 30 plots—6 strips of 5—which were all treated alike but harvested separately, and the crops grown were: 1907, oats; 1908, rye; 1909, barley; 1910, mangolds; 1911, barley. The other field (E_2) was divided into 128 plots—16 strips of 8—and carried oats in 1906, barley in 1907, "seeds" in 1908, and rye in 1909: there was a remarkable oscillation in fertility across this field in one direction, the 1st, 3rd, ... 15th strips consistently giving much higher yields than the 2nd, 4th, ... 16th strips—in fact in the four years the odd numbered strips gave a total yield of 27,817, as compared to 23,383 for the even numbered strips. This oscillation apparently arose as a legacy of the old practice of ploughing in high ridges: the tops of the ridges exhibited greater fertility than the borders of the furrows, so that soil was worked from the former to the latter and the field levelled out: this meant that over the site of the old furrows there was a good depth of rich soil, whilst it was very shallow where the ridges had been. The strips were so arranged as to cover the site of the furrow and of the ridge alternately, with the result noted above: in order to escape this variation, the table was condensed by taking 2 strips together (so that the new strips each included the whole of one of the old "lands") making it an 8 by 8 square. The paper referred to above gives the production for each individual plot as a percentage of the mean yield over the field in that year: the method of enquiry was to suppose that the last year for which figures are given was the actual

experiment, and to calculate how far the knowledge gained by one, two, three, or four, preliminary uniformity trials would be effective in reducing the residual variance (that is, what remained after the variance that could legitimately be taken out of the total by suitable methods of "local control"), by which the significance of the results would be judged.

There appeared to be two printer's errors in the paper from which the figures were taken: with Field A_2 the yields given for the year 1908 add up to 3010 instead of 3000: reference to the Fig. 6 given there seemed to indicate that the excess lay in row 3 and eventually it was decided to reduce plots 3 *c* and 3 *f* to 96 and 84 respectively: again, with Field E_2 in 1908, column 10 sums to 791 instead of 786 as shown: reference to Fig. 13 indicated that the yield of plot 10 *g* should probably have been 92 instead of 97. These two slight changes in the data could, of course, have no appreciable effect on the results.

Field A_2. The following table shows the arrangement of the plots on this field, together with their yields in the supposedly experimental year (1911): the thick lines divide the area into 5 blocks of 6 plots each, suitable for testing six different treatments. These would be assigned to the particular plots wholly at random, with the one restriction that each treatment must occur once in each block: the letters show an arrangement arrived at in this way with treatments denoted by the letters A to F—obtained by entering the letters separately on to six cards, and then shuffling them thoroughly for each block, and writing them down in the order in which the cards were found: since these are dummies they are left out in the working, and only referred to later as an example of the way in which previous information would be used.

D 103	A 101	B 95	D 104	C 109	F 115
B 98	C 102	E 97	A 111	D 109	E 104
F 94	E 106	F 96	C 95	B 112	A 94
C 90	B 111	E 100	C 96	F 99	B 86
D 95	A 105	F 101	A 90	E 98	D 84

Using the methods evolved by Dr Fisher (2), the variance in this year was analysed in the following way:

Sum of the squares of the yields on 30 plots = 301,730.

Sum of the squares of the 5 block totals = 1,804,082.

From these the sums of the squares of the deviations were calculated as

$$\text{Total} = S(y - \bar{y})^2 = 301{,}730 - 30 \times 100^2 = 1730.$$

$$\text{Between blocks} = 6S(\bar{y}^1 - \bar{y})^2 = 6 \times \frac{1}{6^2}(1{,}804{,}082 - 5 \times 600^2) = 680{\cdot}333,$$

giving the following analysis:

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log_e S.D.
Between blocks	4	680·333	170·083	13·0416	2·5683
Within blocks	25	1049·667	41·987	6·4798	1·8687
Total	29	1730·000	59·655	7·7237	—

If, then, 1911 stood by itself the variance of 1 plot due to experimental error would be 41·987, and the significance of the differences between means of 5 replicates would be judged by comparing them to $\sqrt{\frac{2 \times 41 \cdot 987}{5}}$. It is seen that the restriction imposed by the block method of local control has been successful in reducing the variance from 59·655 to 41·987: comparing the variance between blocks with that within blocks, $z = 0{\cdot}6996$, which, with $n_1 = 4$, $n_2 = 25$, gives a value of P lying between 0·01 and 0·05, showing that there were real differences between the average fertility of the blocks.

Treating the other years similarly, the variances within the blocks were found to be as follows:

1910	82·947
Mean, 1909–1910	29·343
,, 1908–9–10	37·785
,, 1907–8–9–10...	28·705

The sum of the products of the yields in any of these and the yields in 1911 can be found exactly similarly to the sum of the squares in any one year, and, having subtracted that between blocks from the total, and divided by 25, the following covariances were found within the blocks:

1910 × 1911	+ 38·020
1909–10 × 1911	+ 25·133
1908–9–10 × 1911	+ 31·053
1907–8–9–10 × 1911	+ 26·347

Substituting the 1910 (x) and 1911 (y) figures in the formula given above, we obtain

$$V_{y\cdot x} = V_y - \frac{(\mathrm{Cov}_{xy})^2}{V_x} = 41{\cdot}987 - \frac{(38{\cdot}020)^2}{82{\cdot}947} = 24{\cdot}560.$$

If, however, yields are to be corrected by a linear regression, one more degree of freedom is used up, and consequently we have, finally

$$V_{y\cdot x} = 24{\cdot}560 \times \frac{25}{24} = 25{\cdot}583.$$

Thus the variance of $y - bx$ is some 40 per cent. less than that of y: the precision obtained by correcting for the 1910 yields can be compared to that of 1911 alone by considering the number of replicates that would be necessary to reduce the variance of the mean to the same point in each case: these are clearly in the proportion 25·583 : 41·987, that is as 1 : 1·642.

The full results obtained with this field were as follows:

Trial years (x)	$V_{y\cdot x}$	Relative precision	Gain in precision
—	41·987	1·000	—
1910	25·583	1·642	0·642
1909–10	21·311	1·969	0·327
1908–9–10	17·152	2·445	0·476
1907–8–9–10	18·547	2·262	−0·183

There is a progressive lowering of the value of $V_{y\cdot x}$ as one, two and three previous years are taken into consideration, until a point is reached at which the experiment is nearly $2\frac{1}{2}$ times as exact as if no previous uniformity trials had been carried out: the inclusion of 1907 does not, however, improve on this, giving, in fact, a slightly higher value. The year 1909 is of some special interest as then the field carried the same crop (barley) as in the supposedly experimental year: taking only that one into consideration $V_{y\cdot x}$ was found to be 35·904, so that a different crop in the preceding year was a much better basis for correction than the same crop two years before.

Field E_2. By putting 2 strips together as mentioned above, the plots in this field were reduced to 64, and were in the form of an 8 by 8 square: with such plots an experiment might be set out in the form of a Latin square, or simply by dividing into 8 blocks of 8 plots each, and the efficacy of previous uniformity trials was tested under both of these arrangements. Taking the Latin square first, the simple addition of the squares of the 1909 yields gave the following:

64 plots	2,566,204
Totals of 8 columns	20,517,636
Totals of 8 rows	20,482,768

Then, since the mean plot yield was 200 and the mean total yield of rows and columns 1600, we have the following as the contributions to the sum of the squares:

$$\text{Total} = 2{,}566{,}204 - 64 \times 200^2 = 6204$$
$$\text{Columns} = \tfrac{1}{8}(20{,}517{,}636 - 8 \times 1600^2) = 4704{\cdot}5$$
$$\text{Rows} = \tfrac{1}{8}(20{,}482{,}768 - 8 \times 1600^2) = 346$$

giving the following analysis

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log_e S.D.
Between columns	7	4704·5	672·071	25·9244	3·2553
Between rows	7	346·0	49·429	7·0306	1·9503
Error	49	1153·5	23·541	4·8519	1·5794
Total	63	6204·0	98·476	9·9235	—

The effect of correcting y for x by linear regression on the variance ascribable to experimental error, in the case of this arrangement on this field, was as follows:

Trial years (x)	$V_{y \cdot x}$	Relative precision	Gain in precision
—	23·541	1·000	—
1908	23·129	1·018	0·018
1907–8	23·737	0·992	− 0·026
1906–7–8	23·981	0·981	− 0·011

It is clear that in this field the plots did not tend to keep their relative yield positions from year to year, so that uniformity trials could serve no useful purpose to correct the yields under experiment: there was in fact a slight loss in precision in the last two cases, which arose by the elimination of one more degree of freedom, for if the covariance is zero, $V_{y \cdot x}$ will obviously be $V_y \times \frac{49}{48}$.

In the above case a large part of the sum of squares was taken out in the columns, so that it would appear possible that previous information might have been valuable if the experiment had been planned on the block system, though that design itself would not have been so efficient: with 8 blocks of 8 plots each, however, there was again no reduction of variance by correction for previous yields:

Trial years (x)	$V_{y \cdot x}$	Relative precision	Gain in precision
—	42·571	1·000	—
1908	41·106	1·035	0·035
1907–8	42·088	1·011	− 0·024
1906–7–8	42·989	0·990	− 0·021

It will be noticed that the variances are much larger in this case, showing that the Latin square method of local control would be much more effective than that of blocks—though 2 strips (2 "columns")

were taken together there yet remained a large variation across that direction.

With the original 128 plots of this field it is unlikely that any experiment would have been laid down with no local control, either by strips or by blocks: if, however, treatments had been completely randomised over the whole field, so that the fertility oscillation in the columns could not have been taken out, the experiment would have lost much in precision, but a considerable proportion would have been retrieved by taking the regression on previous uniformity trials into account: for all the deviations caused by soil variations would have gone into error, and, as the relative yields of the plots in the odd numbered columns were consistently high, whilst those in the even numbered columns were low, a large part of that variance would be eliminated in calculating $V_{y \cdot x}$ from V_y. This serves to emphasise the point that uniformity trials will be more effective in increasing precision in subsequent experiments on very irregular soils, and where local fluctuations are not adequately controlled by the design of the experiment.

Experimental trial.

Although preliminary uniformity trials would be futile on Field E_2 (that is, if reasonable experimental methods were adopted), they would give valuable information on Field A_2, as it appears that there were variations between individual plots (as distinct from those between blocks or strips) of a more or less constant nature—though as to whether this was a matter of plant food, soil texture, drainage, etc., we have no information. The greatest reduction of the variance due to error was given by correction from the yields in the previous three years, so that it was thought desirable to see exactly how the inclusion of this regression would affect the analysis: the random distribution of six dummy treatments is shown on p. 66. The regression of y on x is given by $\frac{\mathrm{Cov}_{xy}}{V_x}$, which in this case takes the value $\frac{38{\cdot}682}{41{\cdot}84645}$ or 0·92438. The various treatments gave:

	Mean plot yield (y)	Mean yield of same plots in 1908–9–10 (x)	($y - bx$)
A	100·2	98·2	9·4
B	100·4	98·8	9·1
C	98·4	100·47	5·5
D	99·0	100·2	6·4
E	101·0	103·8	5·0
F	101·0	98·53	9·9

For actual yields the greatest difference is between E or F and C, and amounts to 2·6: with corrected yields it is seen that E was favoured

by the soil allotted to it, and that it actually evoked the least response, differing from F by 4·9.

Neglecting, at first, the regression, the analysis of variance (of y) is as follows:

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log_e S.D.
Between blocks	4	680·333	170·083	13·0416	2·5683
Between treatments	5	28·800	5·760	2·4000	0·8755
Error	20	1020·867	51·0435	7·1445	1·9663
Total	29	1730·000	59·655	7·7237	—

The random distribution of treatments has been peculiar in giving such small differences: comparing the treatment variance with that due to error, $z = -1{\cdot}0908$, which, with $n_1 = 20$ and $n_2 = 5$, gives a value of P lying between 0·01 and 0·05, showing that such evenness would be given by chance less than 5 times in 100: since practically nothing is taken out by treatment, and 5 degrees of freedom are sacrificed to it, the variance due to error is raised.

The variance of $(y - bx)$ may be determined directly by preparing a fresh table, giving instead of the experimental yield of each plot the value of $(y - bx)$, and by obtaining and analysing the sum of the squares from these new figures. Alternatively it may be derived from tables already calculated for the analysis of the variance of y, and of x, and of the covariance of xy: each of these will contain four rows—one showing the variance (or covariance) between blocks, one between treatments (these of course will always be purely hypothetical in the case of x), one for experimental error, and one for the total. For the compound observation $(y - bx)$ we have

$$S\,(y - bx)^2 = S\,(y^2) - 2bS\,(xy) + b^2S\,(x^2),$$

so that the new table can be constructed by applying this formula to the other three tables, row for row all through. Since $(y - bx)$ contains one statistic (b) already obtained from the data, the total number of degrees of freedom will be reduced from 29 to 28, and, as b has been calculated from the figures for error, this one degree of freedom will be taken from that row. In this way the following analysis of the variance of $(y - bx)$ was obtained:

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log_e S.D.
Between blocks	4	531·133	132·783	11·5230	2·4444
Between treatments	5	115·854	23·171	4·8136	1·5715
Error	19	305·727	16·0909	4·0113	1·3890
Total	28	952·714	34·0255	5·8331	—

In this case our random distribution of treatments shows no peculiarities, the treatment variance agreeing closely with that due to error.

The comparative efficiency in the two cases can be seen from the following:

	Without regression	With regression
Variance of 1 plot	51·0435	16·0909
Variance diff. of means of 5 plots	20·4174	6·4364
S.E.	4·5186	2·5370

A table of t shows that $P = 0{\cdot}01$ when $t = 2{\cdot}845$ with 20 degrees of freedom, and when $t = 2{\cdot}861$ with 19 degrees of freedom, so that a difference of 12·86 would be needed for this standard of significance where the regression was not introduced, but only one of 7·26 when that part of the variance accounted for by it was eliminated. As shown by the values of z in the two cases, the former is not approached by any of the differences between the treatment means, neither is the latter by any of the differences between the mean values of $(y - bx)$: this was to be expected since the treatments were dummies, but had real treatments been carried out it will be seen that the use of the regression would have materially increased the exactitude of the comparison—in fact under the latter conditions 6 replicates would have provided as precise information as 19 would have done if the experimental year gave our only measure of the productivity of the plots. This present insertion of hypothetical treatments into the data shows a greater increase in precision than indicated by the value of $V_{y \cdot x}$ on p. 68, where the relative accuracy in the case of these three preliminary trials was shown to be as 1 : 2·445: the discrepancy arises in the chance distribution of the treatments over the plots, and the value given there for $V_{y \cdot x}$ is the more definite, for that shows what would be the average figure, if a large number of trials with dummy treatments, such as the present, were carried out.

In this instance the gain is very considerable, and it is possible that under certain circumstances (*e.g.* with a restricted area, or where little assistance was available at any one time) it might repay the labour of three years uniformity trials, even though it would increase the work four-fold and the precision but little more than three-fold: such a result must not however be expected in all cases, for with Field E_2, as shown above, the yields under previous trials would be quite ineffective as a basis for correction. The final decision of this question of the value of uniformity trials in this direction must await the analyses of a number of series of uniformity trials carried out on other fields, and it will vary

in each particular case according to the degree of soil heterogeneity of the experimental area, and the adequacy of the methods of local control that are adopted: it is thought however that the present results, meagre though they are, suffice to indicate that in some cases appreciable advances might possibly be made along this line.

SUMMARY.

The question attacked is whether soil variations are sufficiently constant from year to year to give useful corrections to the yields of experimental plots from their yields under previous uniformity trials, and the data investigated were the published results of uniformity trials carried out on two fields at Aarslev (Denmark) between 1906 and 1911. In one case the plots did tend to keep constant in their relative yields, and the precision of an experiment would be increased by nearly 150 per cent. if the regression on the mean yield in the three previous years were used: with the other field, however, the plots showed no constancy in yield (when the variation due to strips was taken out as in modern experimental methods), and consequently previous uniformity trials could give no assistance.

The work described here was done whilst I was enjoying the hospitality of the Rothamsted Laboratory: it gives me much pleasure to acknowledge my great indebtedness to Dr R. A. Fisher, F.R.S., who suggested the problem, and guided my unsteady steps throughout.

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(*Received September 24th*, 1929.)

SULPHUR AS A SOIL FUNGICIDE AGAINST THE POTATO WART DISEASE ORGANISM.

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(With Eight Text-figures.)

Introduction.

The variability in the toxicity of sulphur, when used as a fungicide against the organism that causes the Wart Disease of Potatoes, has formed the subject of a number of earlier papers (2, 10, 11, 12) and led in 1926 to an investigation in the laboratory into the underlying causes. The acidity that results from the oxidation of the sulphur to sulphuric acid, a process which is known to complete itself in soil in a few weeks, having been found to be insufficient to account for the fungicidal action (2, 10, 12), the idea suggested itself that some other compound, formed as a result of the interaction of sulphur and soil, might be responsible. In assessing the importance of any such compound, account has to be taken both of its concentration and of its degree of toxicity. At the outset, therefore, the investigation proceeded along two lines: (1) the exploration of the chemical changes undergone by sulphur in soil, and (2) the determination of the toxicities of compounds formed or at all likely to be formed under these conditions. The second part of the problem has already been the subject of a communication (12).

The first-named part of the investigation, viz. the exploration of the chemical changes undergone by sulphur in soil, which forms the subject of this paper, proved much more difficult. Experiments of as simple a nature as possible were carried out to determine what kinds of compounds are formed when sulphur is incorporated with soil, and to get some idea of the conditions favouring the formation of any such compounds as happened also to be toxic to the Wart Disease fungus. These qualitative, or only partially quantitative, experiments were to have been preliminary to more carefully planned quantitative ones, but owing to the writer's transference to another sphere of work even the

[1] The work described on pp. 85–92 was carried out after the writer had taken up his new appointment.

preliminary experiments had to be somewhat curtailed. Nevertheless the results obtained, so far as they go, do seem to throw some light on the problem and do at least suggest a possible mode of action of sulphur as a soil fungicide, hence they are put on record here. Compounds which it seems at all likely would be formed as a result of the interaction between sulphur and soil fall conveniently into four main classes:

(i) Sulphuretted hydrogen and other sulphides.

(ii) Compounds intermediate in state of oxidation between sulphur and sulphuric acid.

(iii) Sulphuric acid and sulphates.

(iv) The persulphuric acids and their salts, together with hydrogen peroxide and ozone.

These four classes are readily distinguished chemically; sulphuric acid and sulphates alone give an immediate precipitate with barium chloride, whereas members of the first two classes give such a precipitate only after preliminary treatment with strong oxidising agents; members of the fourth class alone give a blue coloration with acidified potassium iodide and starch. The first step, therefore, was to seek evidence of the formation of any members of these classes of compounds under conditions as similar as possible to those in the field. The tests were carried out mainly on aqueous extracts of the soil.

EXPERIMENTAL METHOD.

Soil. Most of the experiments were carried out mainly with two kinds of soil:

1. *Ormskirk* soil, taken from the field at the Potato Testing Station of the National Institute of Agricultural Botany. This was a typical black, peaty, sandy potato soil.

2. *Rothamsted* soil, taken from the electro-culture enclosure. This soil was a stiff clay, containing numerous small pieces of calcium carbonate.

The soil in each case was allowed to dry under cover until it could readily be crumbled, it was then rubbed through a 2 mm. sieve. The portion passing through was thoroughly mixed and moistened with water by means of a spray until a handful on being compressed stuck together, but on gentle rubbing with the finger disintegrated again into its original particles. Plants grow readily in such a soil. After thorough mixing a quantity was weighed out and spread in a uniform layer of about $\frac{1}{2}$-inch thickness on a clean sheet of paper. The requisite quantity of finely ground and sifted sulphur was then distributed as uniformly

as possible through a "100 mesh" sieve over the soil. After a preliminary mixing by hand the soil-sulphur mixture was transferred through a 2 mm. sieve to another piece of paper, care being taken that each charge of the sieve consisted of portions from all parts of the heap. This sifting and mixing process was repeated at least six times.

The prepared soil-sulphur mixture was usually weighed out in 100 gm. lots and put into glass test-tubes placed in constant temperature baths at 0°, 15° and 30° C. respectively, the ends of the tubes being either open to the air or lightly plugged with cotton wool. The moisture content was made up periodically by dropping water slowly from a pipette on to the mixture, care being taken not to cause the particles to run together.

Methods of soil extraction. Three methods of extraction of the soil-sulphur mixture were used.

1. The 100 gm. sample was placed in a glass tube provided at the lower end with a rubber stopper through which passed a No. L. 1 Chamberland filter candle. Water was added, the upper end of the tube then closed with a rubber stopper and the whole thoroughly shaken by hand. The candle was connected to a filter pump through a flask, and the upper rubber stopper removed. This method gave a small quantity of extract within a few minutes of the wetting of the mixture, and it was convenient for use when speed was essential. It was, however, inconvenient for quantitative work, because the rate of filtration slowed down as the pores of the candle became clogged, and unless some salt was added with the second lot of extracting water the clay became deflocculated and rendered a second filtration very slow indeed. (See Fig. 1.)

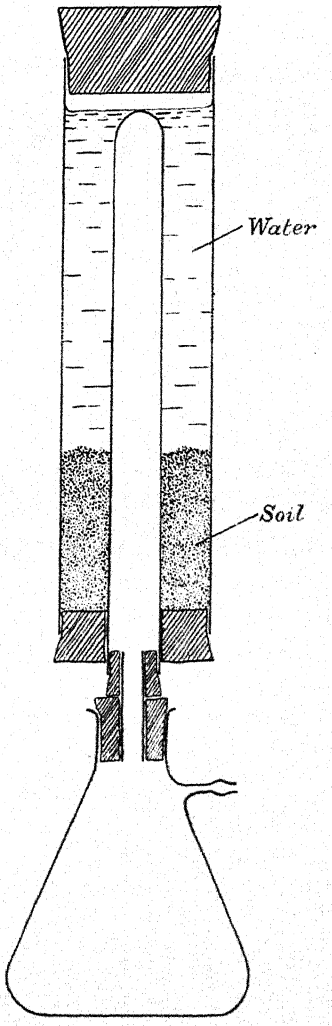


Fig. 1. Apparatus No. 1 for obtaining rapidly a small quantity of extract from soil.

2 (*a*). The 100 gm. sample of soil-sulphur mixture was placed in a Soxhlet extraction thimble standing in a glass funnel, and water was allowed to drop on it at such a rate that it moved from crumb to crumb by capillarity without the intervening air-spaces becoming water-logged. In this way the most concentrated soil extract was obtained, and it was perfectly clear. 5 c.c. of normal sulphuric acid added to and mixed with 100 gm. of the soil-sulphur mixture could be extracted completely in about 12 hours, as may be seen from the following figures which are represented graphically in Fig. 2.

	Volume in ml.	≡ ml. *N*/10 NaOH	Total sulphur extracted by 1 ml. water %
1st percolate	50	13·3	0·519
2nd ,,	70	24·3	0·678
3rd ,,	50	12·2	0·471
4th ,,	60	1·4	0·046
5th ,,	50	0·0	0·00
		51·2	

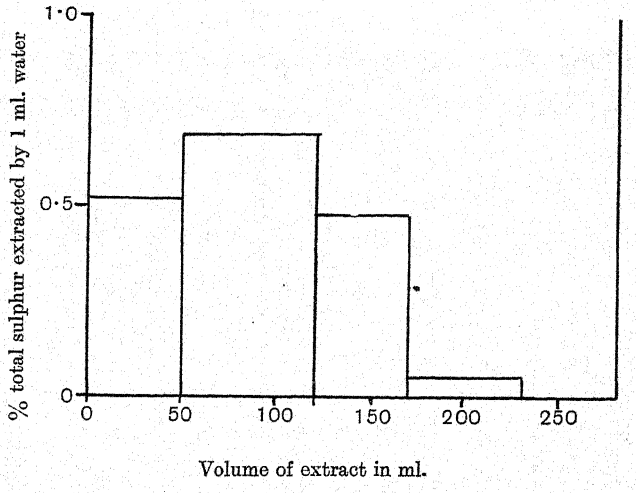


Fig. 2. Amounts of sulphur extracted per ml. water by successive portions of water.

Sulphuric acid added ≡ 51·25 ml. *N*/10 NaOH. Hence 230 ml. water extracted all the sulphuric acid from 100 gm. soil.

2 (*b*). For extracting larger quantities use was made of an elongated Büchner funnel, the elongation consisting of a glass tube of the same diameter as the funnel and fixed thereto by means of surgical rubber tape.

3. The apparatus shown in Fig. 4 was used to obtain extracts under rather less aërobic conditions than in the last method.

Estimation of sulphur in soil.

Extraction. Acetone was found unsatisfactory as a solvent, because it extracted from soil a dark gummy substance which, on subsequent

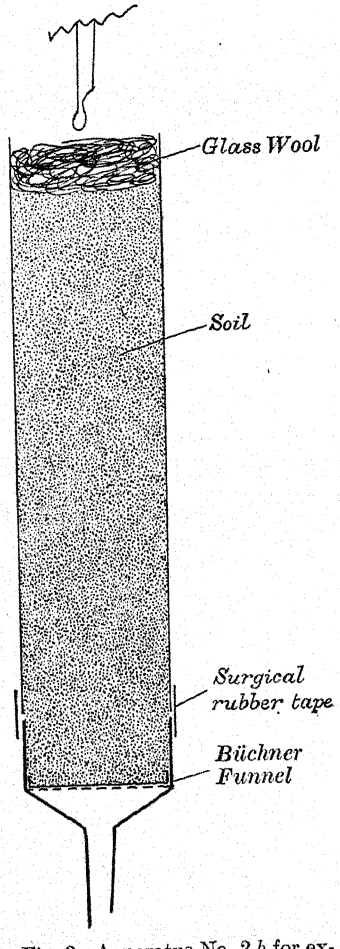


Fig. 3. Apparatus No. 2 *b* for extracting larger quantities of soil.

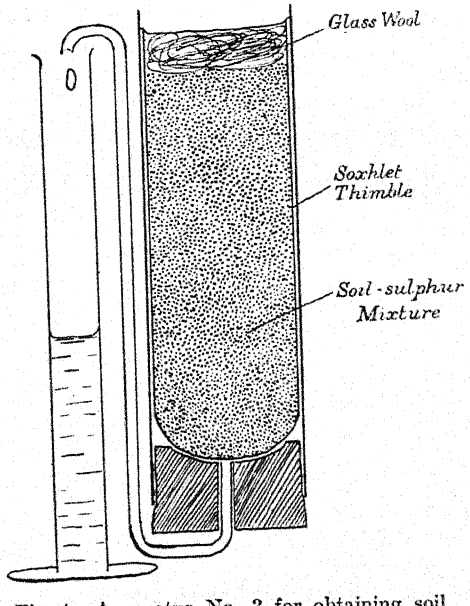


Fig. 4. Apparatus No. 3 for obtaining soil extract under partially aërobic conditions.

oxidation of the extracted sulphur with bromine and nitric acid or by other means, gave a yellowish resinous substance highly resistant to oxidation. Carbon tetrachloride, however, extracted the sulphur almost free from the contaminating gummy substance, even from Ormskirk soil, which was particularly troublesome when acetone was used.

Estimation. The carbon tetrachloride extract was evaporated to

dryness on the water bath. The neck of the flask was closed with a ground-in tap funnel (the joints were ground so that the flasks would fit either the Soxhlet extractors or the ground-in tap funnel), Fig. 5. The flask was evacuated and the tap closed. For 0·1 gm. sulphur 10 c.c. $N/1$ caustic soda was added through the tap without admitting any air and the flask was replaced on the water bath and left there until all the sulphur had dissolved. In this way any loss of volatile sulphur compounds was avoided. The oxidation was carried out by means of hydrogen peroxide, or, more readily, by potassium bromate and hydrochloric acid, as in the method of Treadwell and Mayr (13). The sulphate formed was estimated by one of the recognised methods.

Absence of sulphuretted hydrogen and sulphides.

In all the experiments described tests were made for sulphuretted hydrogen and sulphides in three ways: (*a*) by hanging a lead acetate paper just above the soil-sulphur mixture, (*b*) by moistening such a paper with the extract, and (*c*) by placing a little soil-sulphur mixture on the paper and carefully dropping enough water on the soil to cause it to adhere to the paper. The paper invariably remained white; and numerous other experiments not referred to also gave the same negative results. The temperature varied from 0° to 30° C.; the soil varied from the typical Rothamsted field soil through a rich allotment soil from Rothamsted to the peaty sandy Ormskirk soil, and aëration varied down to complete water-logging for more than a week on end. It seems unlikely, therefore, that sulphuretted hydrogen or other sulphides are ever formed under field conditions in sufficient quantity to have any appreciable toxic action on the Wart Disease fungus, especially in view of their low degrees of toxicity.

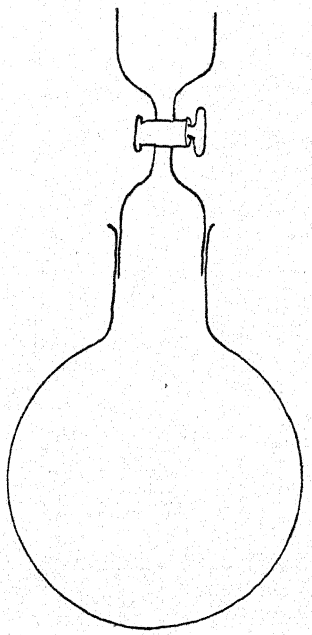


Fig. 5. Oxidation flask for method of Treadwell and Mayr.

Absence of persulphates, etc.

Numerous tests made on soil-sulphur mixtures kept under even more widely varying conditions gave no clear indications of the formation of

any substance able to liberate iodine from slightly acidified potassium iodide; whenever the blue starch-iodide colour was given in a paper test over sulphur-treated soil or in air aspirated through such soil a similar colour was also given when untreated soil was substituted for the sulphur-treated soil. In no single experiment carried out at temperatures likely to be met with in the soil (say up to 30° C.) was there any difference in the behaviour of untreated and sulphur-treated soils as judged by the intensity of the blue colour developed. In spite of the high toxicity of persulphuric acid it seems unlikely that enough could have been formed under these conditions to explain the toxicity of the sulphur. In experiments carried out entirely in solution on soil extracts the results were more definitely negative. A blue colour was never obtained on acidifying and adding potassium iodide and starch solution to a colourless soil extract.

Compounds of classes (i) and (iii) therefore, are not further dealt with in this communication.

Evidence for the formation of compounds intermediate in state of oxidation between sulphur and sulphuric acid.

The following compounds, among others, belong to this class: thiosulphuric acid, $H_2S_2O_3$; pentathionic acid, $H_2S_5O_6$; tetrathionic acid, $H_2S_4O_6$; trithionic acid, $H_2S_3O_6$; dithionic acid, $H_2S_2O_6$; sulphurous acid, H_2SO_3; and the salts of these acids.

The experimental procedure in testing for the accumulation of these substances in soil-sulphur mixtures was to treat the soil extract with excess of barium chloride, spin down the barium sulphate precipitate on the centrifuge, then treat the solution with potassium chlorate and hydrochloric acid, when a further precipitate would indicate the presence of one or more of these compounds. (It has already been stated that tests for sulphides were uniformly negative.) A number of preliminary experiments of this kind indicated that in the Rothamsted soil to which sulphur had been added, appreciable quantities of the sulphur were oxidised in the course of a few days to compounds which, on oxidation with chlorine or other strong oxidising agents, gave sulphate. On a number of occasions tests were made for thiosulphate and sulphite by precipitating the sulphate with excess of barium chloride, removing the precipitate and titrating with iodine. In all experiments except one 2 drops of decinormal iodine solution were sufficient to give the "starch-iodide" blue; in the one experiment, as will be seen later (p. 82), 0·2 c.c. was necessary. This evidence, though perhaps suggestive,

is insufficient to establish the presence of thiosulphate or sulphite. The fact that the addition and slight combination of iodine was never followed by a further precipitate of barium sulphate proved that if either of these two compounds was present it was thiosulphate, which, as is well known, gives tetrathionate with iodine, whereas sulphite forms sulphate. Whenever an appreciable further precipitate was formed after treatment with a strong oxidising agent, another portion of the solution always gave a white, or sometimes even a yellow, precipitate of sulphur, on the addition of 20 per cent. caustic soda. The solution also gave the other tests characteristic of polythionates. Hence the solutions definitely contained pentathionate and possibly other polythionates, though the lower polythionates cannot be detected by any known qualitative test in the presence of pentathionate. The characterisation of the polythionates in the presence of each other can be done only by quantitative means.

The few results of some work along quantitative lines did not justify the drawing of definite conclusions.

A few of the experiments proving the presence of polythionates in sulphur-treated soil are given in more detail below. In most of these the amount of sulphur added was chosen to correspond with a dressing of 1 ton per acre in the field, *i.e.* 0·1 gm. sulphur was added to 100 gm. of the damp soil.

EXPERIMENTAL RESULTS.

I. *Experiment begun*, 28. viii. 26.

Rothamsted soil, 5000 gm., mixed with 5 gm. sulphur (as used at Ormskirk in 1925). Temperature, 30° C.

Untreated soil. An extract of 100 gm. of soil was made by means of apparatus 1. The filtrate was perfectly clear and neutral in reaction. It gave only a barely perceptible turbidity with barium chloride and with benzidene; hence it was practically free from sulphate. After oxidation with hydrogen peroxide or potassium chlorate and hydrochloric acid and subsequent addition of barium chloride, or benzidene, the solution became only very faintly turbid; hence it was free from all but minute traces of all sulphur compounds.

Soil and sulphur mixture. The same results as above were obtained with the aqueous extract made from the sulphur-treated soil immediately after the sulphur was added; hence there was no appreciable immediate reaction between the soil and sulphur.

7. ix. 26 (10 *days after beginning of experiment*).

Tube No. 37 percolated by method 2 and sulphate determined by Raschig's benzidine method.

1st 80 c.c. of percolate ≡ 5·49 c.c. *N*/10 NaOH
Next 80 c.c. of percolate ≡ 0·00 „ „
5·49 „ „
≡ 0·018 gm. sulphur as sulphate,
i.e. 18 per cent. sulphur oxidised to sulphate.

8. ix. 26. *Test for forms of sulphur other than sulphur and sulphate.*

Two tubes of soil were percolated by method 2, till the percolate no longer gave a precipitate with barium chloride. Each percolate was treated with excess of barium chloride and the precipitated barium sulphate centrifuged off, washed and weighed.

Weights of barium sulphate: (1) 0·0988 gm., (2) 0·1069 gm. The centrifugate, which was perfectly clear, was boiled with hydrogen peroxide. There was a further small precipitate of barium sulphate, indicating that the solution contained some oxidisable sulphur compound soluble in water.

Weights of barium sulphate: (1) 0·0458 gm., (2) 0·0467 gm. Hence in 11 days at 30° C. about

14 per cent. of the sulphur was in the form of sulphate,
6 per cent. of the sulphur was present as some compound oxidisable by hydrogen peroxide to sulphate,

and the remaining

80 per cent. presumably unchanged.

II. *Experiment begun*, 21. i. 27.

Rothamsted soil, 0·2 per cent. sulphur. Temperature, 30° C.

2. ii. 27 (12 *days after beginning of the experiment*).

100 gm. of soil percolated by method 2 and oxidisable sulphur estimated by the method of Treadwell and Mayr.

1st percolate	50 c.c.	(12.30–6 p.m. = 5½ hr.)	≡ 7·00 c.c. $N/10$ $Na_2S_2O_3$.	
2nd „	40 „	(during night)	≡ 2·55 „	„
3rd „	50 „	(10 a.m.–12.30 p.m. = 2½ hr.)	≡ 1·35 „	„
4th „	50 „	(12.30–5.30 p.m. = 5 hr.)	≡ 1·02 „	„
5th „	50 „	(during night)	≡ 0·22 „	„
			12·15 „	„

12·15 c.c. 0·998 $N/10$ $Na_2S_2O_3$ ≡ 0·0097 gm. S_5O_6'' sulphur (or about 0·5 per cent.),

or 0·015 gm. S_5O_6'' sulphur (0·7 per cent.).

8. ii. 27 (20 *days after beginning of experiment*).

Extracts of two separate tubes made by method 3.

No. 1. 1st 150 c.c. extract treated by Treadwell and Mayr method

≡ 7·3 c.c. 0·998 $N/10$ $Na_2S_2O_3$,
or 0·0058 gm. S_5O_6'' sulphur, or about 2·9 per cent.

No. 2. 1st 150 c.c. extract decolorised 1 drop but not 2 drops $N/10$ iodine.

Hence no appreciable quantity of S_2O_3'' or of SO_3'' was present.

Two tubes extracted in the same way as the above two but more slowly; the extracts being united, mixed and divided into two equal parts.

1st half decolorised 1 drop but not 2 drops of $N/10$ iodine.

2nd half when oxidised in the Treadwell and Mayr apparatus

≡ 9·1 c.c. $N/10$ $Na_2S_2O_3$,
≡ 0·0073 gm. S_5O_6'' sulphur, or **3·6** per cent.

9. ii. 27 (21 *days after commencement of the experiment*).

Two lots of 500 gm. sulphur-treated soil extracted by method 2 *a*.

Polythionate sulphur:

(1) ≡ 75·1 c.c. *N*/10 bromine or 0·06 gm. S_5O_6'' sulphur (6 per cent.);

(2) ≡ 50·0 „ „ 0·04 „ „ (4 per cent.).

(1) 50 c.c. solution ≡ 0·20 c.c. *N*/10 iodine solution;

(2) „ „ ≡ 0·20 „ „

This was the only indication of any appreciable quantity of thiosulphate or sulphite being formed. This evidence is insufficient on which to base the conclusion that a definite amount of thiosulphate or sulphite was present in the extract.

There is definite evidence therefore that in Rothamsted soil kept at 30° C. pentathionate (accompanied possibly by other polythionates) is formed in quantities up to 6 per cent. of the sulphur added. No evidence was obtained in these experiments of the formation of any appreciable quantities of sulphur compounds intermediate in state of oxidation between sulphur and sulphuric acid in Rothamsted soil kept at 15° and 0° C. respectively.

Ormskirk soil. Similar but less extensive experiments were carried out on Ormskirk soil; in none of the experiments was there any evidence of the formation of appreciable quantities of such compounds.

Hence we have definite evidence of only one compound being formed in any appreciable quantity as an intermediate product in the oxidation of sulphur to sulphuric acid, viz. pentathionate; but possibly this was accompanied by other polythionates. None of the polythionates shows any toxicity towards the Wart Disease fungus, however, hence their formation on sulphur-treated soil throws no direct light on the mode of fungicidal action of sulphur; but it does give a hint of a conceivable mode of action. It is well known that polythionates are formed when a thiosulphate is acidified, hence the question arises: was the pentathionate found in the soil solution formed from pre-existing thiosulphuric acid? *i.e.* does the oxidation take place in some such steps as the following:

sulphur → thiosulphuric acid → polythionic acids → sulphuric acid.

Much support may be found for this view in the work on the oxidation of sulphur in soil and other habitats and by micro-organisms in culture fluids reviewed by Joffe(8) and Guittonneau and Keiling(6). Probably all of these changes are brought about mainly by micro-organisms, but chemical evidence, as reviewed by Bassett and Durrant(1), also supports the view that some such series of changes as the above takes place.

The fact that the presence of thiosulphate in appreciable quantities was not definitely established by the foregoing experiments is not evidence against the above view. The amount accumulating in the soil solution will be conditioned both by the rate of its formation from sulphur and by the rate of its change into pentathionate. If, now, the soil be extracted, the sulphur is removed with the soil from the reaction solution and the source of the thiosulphate disappears; but there is no reason for supposing that the change of thiosulphate into pentathionate is interfered with materially; thus there is reason for supposing that the amount of thiosulphate in the solution will decrease immediately the sulphur is separated from it. Hence to test for its formation with any degree of certainty it would be necessary either (*a*) to remove the thiosulphate as an insoluble compound actually from the soil solution itself, (*b*) to convert it into a compound not changed to pentathionate, or (*c*) to arrest all these changes suddenly such as by very low temperature and subsequent extraction with alcohol. An opportunity to carry out such experiments did not present itself; but recently work has been done which has shown that thiosulphuric acid can exist in a free state; a comparison has been made between the degree of toxicity of certain solutions and their content of thiosulphuric acid. This helps to assess the value of the suggestion that sulphur acts as a soil fungicide through the formation of thiosulphuric acid.

The existence in a free state and the degree of stability of thiosulphuric acid.

The existence of thiosulphuric acid in a free state is commonly denied in text books, thus Ephraim(3) states: "the free acid has not been isolated, as it decomposes at once into sulphurous acid and sulphur," but, as already indicated in a former paper ((12), p. 181), even after 12 hours an *S*/1000 acidified thiosulphate solution remained perfectly clear. Even much stronger solutions remain clear for shorter lengths of time. Though acidified thiosulphate decomposes in other ways giving rise to products other than sulphur and sulphur dioxide(1), these facts do rather suggest that the decomposition of free thiosulphuric acid is not instantaneous but requires a definite period of time for completion and that the final equilibrium solution in each case will contain definite amounts of free thiosulphuric acid. Before this idea could be tested analytical methods have had to be modified for the purpose.

The readiest method of estimating thiosulphate is by titration with standard iodine solution, but this method is not applicable unmodified in the presence of sulphite, since sulphite also is oxidised by iodine.

The sulphite may be precipitated and removed, or more simply it may be locked up by treatment with formaldehyde in a compound in which it is not acted upon by iodine; the thiosulphate may then be titrated with iodine (assuming sulphides to be absent, as in fact they were in all the solutions examined). The necessary conditions for concentrated solutions were worked out by Kurtenacker (9). Certain refinements, however, are necessary before the method is applicable to dilute solutions. The modified method will be described before giving the results obtained by it.

Estimation of thiosulphate in presence of sulphite by a modification of Kurtenacker's method.

The essence of Kurtenacker's method is to neutralise the solution to phenol phthalein with ammonia to remove any strong acids, add an excess of formaldehyde, then render acid with acetic acid and titrate immediately after acidification with iodine solution. Under these conditions the sulphite is rendered almost completely inactive towards iodine, the excess formaldehyde has no measurable effect on iodine and any formaldehyde thiosulphuric acid formed is sufficiently unstable to allow of rapid titration of the thiosulphate part of it.

The method exactly as recommended by Kurtenacker was found satisfactory for the relatively concentrated solutions with which he worked, but in more dilute solutions it became inapplicable. The following modifications were found necessary or considered desirable.

Excess of potassium iodide. For iodine-thiosulphate titrations in $N/10$ solution the amount of the excess of potassium iodide added does not matter within limits, though even in such concentrated solutions a large excess causes the colour with starch to be brown rather than blue and to fade rather rapidly making the end-point somewhat indefinite. No experimental evidence could be found for the desirability of the addition of any potassium iodide besides that usually contained in the iodine solution. As will be seen later, the error, through omitting to add further potassium iodide, is only a small fraction of a drop of $N/10$ iodine solution. The influence of the concentration of potassium iodide in the solution titrated is well seen by determining the amount of standard iodine solution necessary to give a definite colour in presence of varying amounts of potassium iodide.

Each solution contained 50 ml. water, 1 ml. 0·5 per cent. starch, 1 ml. glacial acetic acid.

ml. 10 % KI added	0	1	5	10
1 drop $N/100$ iodine	No colour	Pure blue	Not pure blue	Reddish blue
2 drops „	Faint pure blue	—	—	—
3 drops „	Definite pure blue	—	—	—

Thus 2 drops of $N/100$ iodine are sufficient to give a definite though faint coloration in 50 ml. solution and 3 drops an easily recognised blue colour, without the addition of any potassium iodide other than that in the iodine solution. The addition of 1 ml. 10 per cent. potassium iodide to 50 ml. solution (*i.e.* 0·2 per cent. KI) was sufficient to cause 1 drop of $N/100$ iodine solution to give a definite pure blue colour. The colour became more and more reddish as more potassium iodide was added, becoming brown and finally practically disappearing when large excesses were added.

With $N/1000$ iodine using the same solution as the above the following results were obtained:

ml. 10 % KI added	0	0·25	0·5	1
1 drop $N/1000$ iodine	Colourless	Colourless	Colourless	Colourless
5 drops "	"	Light blue	Light blue	Light blue
10 drops "	"	Blue*	Blue*	Blue*
25 drops "	Just perceptible colour	—	—	—
30 drops "	Blue*	—	—	—

Solutions marked (*) were of approximately the same colour. On the further addition of 5 ml. 10 per cent. KI the blue colour became tinged with red but was hardly any deeper.

Hence the presence of 0·2 per cent. potassium iodide was sufficient to cause 1 drop of $N/100$ iodine, to give an easily distinguishable blue colour and 5 drops of $N/1000$ iodine to give a definite light blue colour. This concentration was accepted as the best for the present purpose.

Excess of formaldehyde. Experiments were carried out to determine the minimum amounts of formaldehyde necessary to render inactive towards iodine the various amounts of sulphite likely to be dealt with. Thus using 1 ml. 35 per cent. formaldehyde in 50 ml. $S/200$ sodium sulphite (+ 1 ml. glacial acetic acid, 1 ml. 10 per cent. KI, 1 ml. 0·5 per cent. starch), 1 drop of $N/100$ iodine gave a definite colour. With quantities of formaldehyde less than 1 ml. more than 1 drop of $N/100$ iodine was necessary.

In order to determine whether this amount of formaldehyde had any serious effect on the iodine titration under the experimental conditions the following solutions were made up:

A	*B*
50 ml. water	50 ml. water
1 ml. 35 % formaldehyde	
1 ml. 10 % KI	1 ml. 10 % KI
1 ml. glacial acetic acid	1 ml. glacial acetic acid
1 drop $N/100$ iodine	1 drop $N/100$ iodine
1 ml. 0·5 % starch solution	1 ml. 0·5 % starch solution

The colour of solution *A* was visible even without using a beaker of pure water for comparison; hence the difference due to an excess of 1 ml. 35 per cent. formaldehyde was less than 1 drop of *N*/100 iodine.

The colour of solution *A*, however, was fainter than that of solution *B*; hence even this small amount of formaldehyde had some small effect on the iodine, so it was considered undesirable to increase the amount more than necessary. This amount was found satisfactory for *S*/20, *S*/100 and *S*/1000 solutions.

The modified method, therefore, is as follows: to 25 or 50 ml. solution are added (in the following order) enough ammonia to render neutral to phenol phthalein, 1 ml. 35 per cent. formaldehyde, 1 ml. 10 per cent. potassium iodide and 1 ml. glacial acetic acid; immediately after the addition of the acetic acid the solution is titrated with iodine.

The following results demonstrate the degree of accuracy readily attainable:

1. 25 ml. *S*/100 $Na_2S_2O_3$ + 25 ml. water + 1 ml. glacial acetic acid + 1 ml. 10 per cent. potassium iodide + 1 ml. 0·5 per cent. starch
   ≡ (12·45), (12·44), 12·49, 12·49 ≡ **12·49** ml. *N*/100 iodine solution.
2. The above solution + 1 ml. 35 per cent. formaldehyde
   ≡ **12·49** ml. *N*/100 iodine solution.
3. Solution 2 with water replaced by 25 ml. *S*/100 Na_2SO_3
   ≡ 12·49, 12·51 ≡ **12·50** ml. *N*/100 iodine solution.

By the time the above analytical method had been worked out it was no longer possible to test the toxicities of solutions simultaneously with their analysis. The best that could be done therefore was to analyse solutions made up to be as similar as possible to certain of those the toxicities of which had already been determined(12). In that paper (p. 181) are given the toxicity figures for a double series of solutions of thiosulphuric acid in the presence of excess of sulphuric acid in the one series and of sulphurous acid in the other series.

When a thiosulphate is acidified a variety of compounds are formed which exist more or less in equilibrium with each other. As is well known, if to such a decomposing compound one of its products of decomposition is added, the decomposition tends to be arrested and so the equilibrium mixture tends to contain more of the undecomposed compound. Thus sulphurous acid being known to be a decomposition product of thiosulphuric acid it might be expected that the substitution of sulphurous for sulphuric acid in the solution of thiosulphuric acid

would result in changes in the equilibrium solution obtained. In particular the relation between any undecomposed thiosulphuric acid and its decomposition products would be upset. That some such modification of the equilibrium mixture was brought about is shown by the fact that

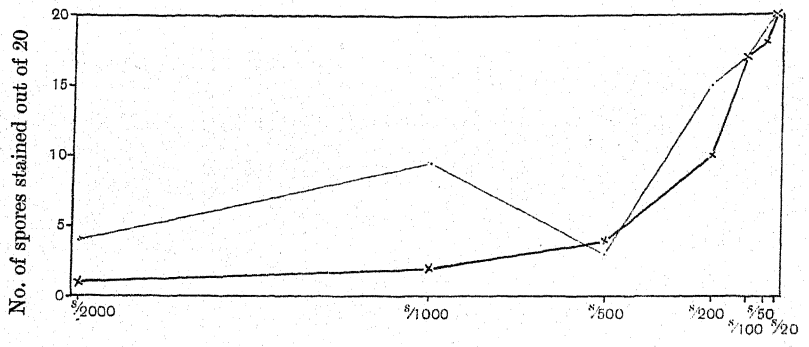


Fig. 6.

Connection between concentration and toxicity of thiosulphuric acid solutions in presence of excess of sulphuric acid ——•——•, sulphurous acid ——×——×. Spores treated for 24 hours. Results taken from Roach and Glynne (12).

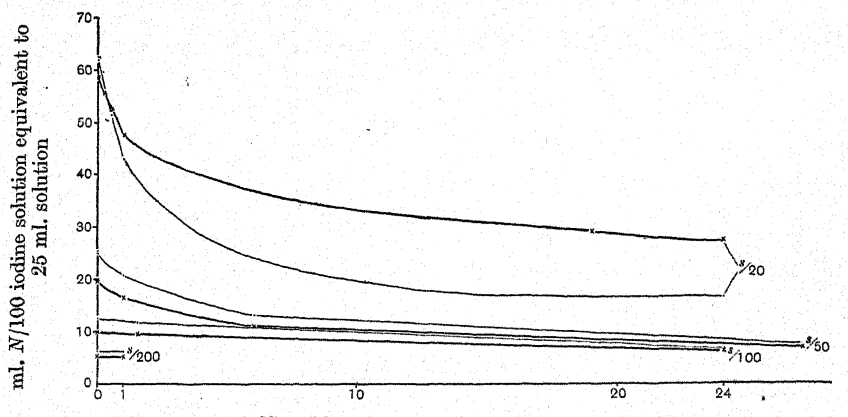


Fig. 7.

Concentration of thiosulphuric acid after varying lengths of time in presence of excess of sulphuric acid ——•——•, sulphurous acid ——×——×.

the separation of sulphur was different in the two series of solutions, hence a comparison of the toxicities of such solutions with their thiosulphuric acid contents might be expected to throw light on the question of the degree of connection between them. As may be seen from Fig. 6 toxicities of corresponding solutions of the two series are the same

within the admittedly large experimental error. Similar solutions were made up and their thiosulphuric acid contents determined after standing for varying lengths of time. The analytical results are shown in Table I and Fig. 7. The figures given are the numbers of ml. $N/100$ iodine solution equivalent to 25 ml. of the solution tested. As a check an aliquot portion of each solution was titrated before the acid was added and in addition some of the solutions were neutralised and titrated immediately after the acid was mixed. It will be seen from Table I that there is no justification for the general belief that thiosulphuric acid is completely decomposed as soon as liberated; for at the end of the few minutes necessary to mix the acid and thiosulphate solutions, pipette off the aliquot portion and neutralise it, less than 1 per cent. of the thiosulphate had disappeared.

Table I. $H_2S_2O_3$.

	S/20		*S*/50		*S*/100		*S*/200	
	Excess H_2SO_4	Excess H_2SO_3	Excess H_2SO_4	Excess H_2SO_3	Excess H_2SO_4	Excess H_2SO_3	Excess H_2SO_4	Excess H_2SO_3
Before mixing	62·4	58·6	25·25	19·2	12·65	9·96	6·265	5·2
Immediately after mixing	61·94	58·4	25·05	—	12·6	—	—	—
¼ hr. after mixing	56·0	55·65	—	—	—	—	—	—
½ hr. „	51·1	52·2	—	—	—	—	—	—
1 hr. „	43·0	47·4	20·01	16·5	—	—	6·21	5·15
1½ hr. „	—	—	—	—	11·9	9·95	—	—
19 hr. „	—	29·0	13·0	11·0	—	—	—	—
24 hr. „	16·5	27·25	—	—	6·9	6·3	—	—
27 hr. „	—	—	7·65	7·0	—	—	—	—
44 hr. „	10·0	—	—	—	—	—	—	—
7 days after mixing	—	—	3·35	2·35	2·7	2·35	2·83	2·8

Next, the increase of stability of the thiosulphuric acid with dilution will be noticed; thus the titration figure for the $S/20$ thiosulphuric acid with excess of sulphuric acid in 24 hours decreased from 62·4 to 16·5 (*i.e.* roughly 26 per cent. left undecomposed), whereas the corresponding $S/100$ solution only decreased from 12·65 to 6·9, *i.e.* 55 per cent. left undecomposed. Results at the end of 7 days lead to the same conclusion. Whereas the figure for the $S/50$ solution decreases from 25·25 to 3·35 (*i.e.* 13 per cent. undecomposed), the $S/200$ solution only decreases from 6·265 to 2·83 (*i.e.* 45 per cent. still undecomposed).

Bearing in mind the fact that the solution in which the excess acid was in the form of sulphurous acid was poorer in thiosulphuric acid at the start than those in which the excess acid was sulphuric acid, the figures show that the substitution of an excess of sulphurous for an excess of sulphuric acid has resulted in a slight increase of stability of the thiosulphuric acid; but taking into account the inaccuracy of the

toxicity figures, this increase in stability is insufficient to affect the toxicity figures appreciably, except perhaps for the $S/20$ solutions; as both of these are completely toxic under the conditions of the test, any such difference cannot show itself.

The toxicity, therefore, varies with the thiosulphuric acid content as far as can be judged from the result of this test, which, as it turns out, is admittedly not a very searching one. Even though the replacement of the excess of sulphuric acid by the excess of sulphurous acid did not result in any great change in thiosulphuric acid content, the difference in sulphur deposition ((12), p. 181) shows that the equilibrium of the solutions was definitely upset in other ways. The toxicity figures following the thiosulphuric ones and not the sulphur deposition make the connection between the toxicity figures and thiosulphuric acid content the more likely to be a causal one. The decomposition of the thiosulphuric acid becomes very slow when it reaches a concentration of about $S/100$ or lower. This solution is almost completely toxic in 24 hours. This fact may explain why $S/20$ thiosulphuric acid remained completely toxic when the spores were exposed for 24 hours even when tested 7 days after the solution was made up, and why an $S/200$ solution had approximately the same toxicity whether tested immediately after the solution was made up, 1 day or 7 days afterwards, as stated on pp. 179 and 180 of the previous paper (12).

We can test still further the idea that sulphur owes its fungicidal action in soil to the formation of thiosulphuric acid; we can see to what extent the minimum toxic concentration of thiosulphuric acid found in the laboratory fits in with the minimum effective dressing of sulphur in the field.

An exposure of Wart Disease spores to an $S/200$ solution of thiosulphuric acid for 10 days results in about 19/20 being killed ((12), p. 178). It has been calculated that a dressing of about 11 cwt. sulphur per acre can free the soil from disease in the ensuing season (10). We can, therefore, say that these two treatments are roughly equivalent in their effects on the disease-producing organism. 1000 c.c. $S/200$ solution of thiosulphuric acid contains

$$\frac{32}{200} = 0{\cdot}16 \text{ gm. sulphur.}$$

11 cwt. per acre is roughly equivalent to 0·055 per cent. on the top 9-inch soil; assuming a 20 per cent. soil mixture content, 1000 c.c. soil solution will contain $0{\cdot}055 \times \frac{100}{20}$

$$= 2{\cdot}75 \text{ gm. sulphur.}$$

For 1000 c.c. of solution to be completely toxic we have seen that it must contain 0·16 gm. sulphur as thiosulphuric acid over a period of 10 days, *i.e.* $\frac{0{\cdot}16 \times 100}{2{\cdot}75}$, or roughly 6 per cent. of the sulphur added.

It is not likely that the fungicidal action of sulphur would ever be over in so short a time as 10 days; if not, the above figure of 6 per cent. must be reduced, but against this must be placed the fact that the thiosulphuric acid has to reach each spore by diffusion in at least the minimum toxic concentration, seeing that it is continually decomposing it must start off at the source, the sulphur particle, at a somewhat high concentration.

After preparing this communication for publication the writer has read the criticisms made by Williams and Young (14) of deductions drawn in an earlier paper (12). They state: "Recent work by Roach and Glynne was interpreted by them as pointing to thiosulfuric acid as a toxic factor. However, since the thiosulfuric acid is a very unstable acid and since the condition of their experiments were such as to insure the presence of polythionic acids, the toxicity which they measured was undoubtedly that of the polythionic acids. Furthermore, the tables given by them show a notable toxicity of pentathionic acid itself when compared with other acids tested."

Facts reported on pp. 85–92 of this paper are a sufficient answer to that part of their criticism which is based on the supposed complete instability of thiosulphuric acid.

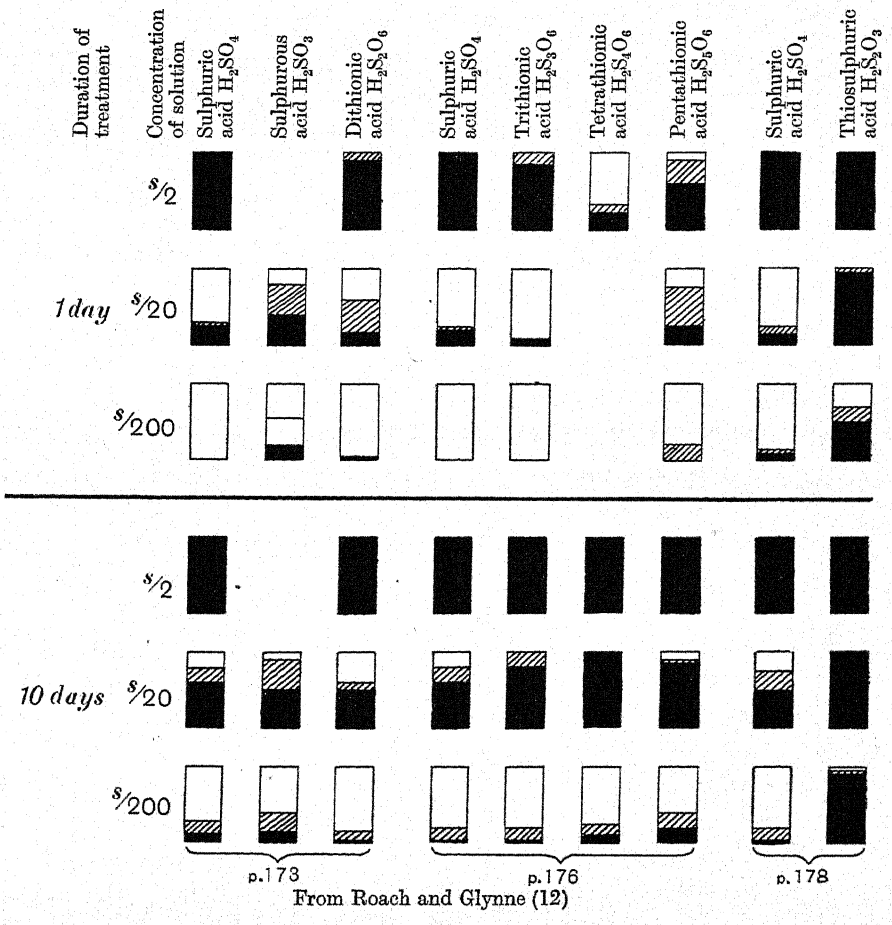
The sample of barium pentathionate used ((12), p. 188) was exceptionally pure (over 90 per cent.), and in making up the pentathionic acid solutions allowance was made for the 10 per cent. impurity which was probably water. The dilute solutions, when made up, were free from all but traces of sulphite and thiosulphate[1] and remained so the whole period of the tests (up to 10 days). The toxicities of these solutions therefore probably were a fair measure of that of pentathionic acid. The present writer cannot agree with Williams and Young's statement: "the tables...show a notable toxicity of pentathionic acid itself when compared with other acids tested" and sees no reasons for departing from the original conclusion ((12), p. 175): "The three polythionic acids were of the same order of toxicity as sulphuric acid" (see Fig. 8 in which the relevant data are reproduced). Even assuming, what may actually be true, that pentathionic acid has a slightly higher toxicity than sulphuric acid, it is definitely less toxic than thiosulphuric acid, the difference being outside the large limit of experimental error of the tests. Whatever the cause of the toxicity of acidified thiosulphate solutions it can hardly be through the formation of pentathionic acid which is of lower toxicity when compared on a sulphur basis.

That thiosulphuric is the factor chiefly, if not entirely, responsible for the toxicities of the sulphur compounds considered (over and above that due to the hydrogen ion) seems to offer the simplest explanation and the one most free from contradictory

[1] Williams and Young give the results of no such tests to establish the purity of the compounds used in their work. The writer found it necessary to prepare many samples and purify repeatedly before one even relatively free from thiosulphate was obtained.

facts and unproven assumptions; hence it is retained unless and until a better one presents itself. The difficulties and uncertainties connected with the chemistry of sulphur compounds make anything approaching a final conclusion at this stage unjustifiable.

As already stated, the above tentative conclusion referred to Wart Disease only and does not necessarily relate to any other fungus.



From Roach and Glynne (12)

Fig. 8. Toxicities of various sulphur acids towards *Synchytrium endobioticum*.

Key to diagram:

- Sporangia stained faintly and therefore alive.
- Sporangia stained to intermediate degree and probably dead.
- Sporangia stained deeply and therefore dead.

DISCUSSION.

It was suggested on p. 83 that the oxidation of sulphur to sulphate in soil-sulphur mixtures may take place in the following stages:

sulphur → thiosulphate → polythionate(s) → sulphate.

There seems little doubt that sulphate is the end-product under a variety of conditions. Pentathionate has been proved in the present investigation to be formed, and to accumulate, in sulphur-treated Rothamsted soil kept at 20° C., but, even if formed under the other conditions tried, it did not accumulate appreciably. These observations have been confirmed by Hobson (see Appendix). He has also shown that thiosulphate when added to soil is changed first into pentathionate and then into sulphate. Of the change of sulphur into thiosulphate, the present investigation offers no more than a mere suggestion (p. 82), but Guittonneau and Keiling(4, 5, 6, 7) proved that thiosulphate accumulated in soil-sulphur mixtures under the conditions of their experiments. Moreover, they showed that the addition of peptone greatly increased the accumulation of this substance, thus proving that the degree of accumulation is affected by the chemical composition of the soil solution.

These facts afford sufficient indications of the sensitiveness of the above series of chemical changes to physical and chemical conditions. They suggest the possibility that under the conditions of 1925 and previous years, when sulphur treatment was effective in controlling Wart Disease in the succeeding crop of potatoes, the oxidation of the sulphur may have taken place in such a way that there was a temporary accumulation of thiosulphuric acid sufficient to make the soil solution toxic to the fungus, whereas under the colder and in other ways different conditions of 1926, when the disease was apparently unaffected, there was little or no accumulation of this substance.

This explanation is the only one put forward at present at all in harmony with the facts; but it cannot be regarded as established unless and until a definite connection has been proved between the amount of thiosulphuric acid accumulating in the soil and the degree of effectiveness of the treatment.

SUMMARY.

(1) Thiosulphuric acid has been shown to exist in a free state.

(2) It is relatively stable in dilute solution; an $M/200$ solution is only half decomposed at the end of 1 day and an $M/400$ solution at the end of 10 days only.

(3) This degree of stability is sufficient to account for the fungicidal action of acidified thiosulphate solutions in terms of the liberated thiosulphuric acid.

(4) It can be calculated that it is only necessary to assume 6 per cent. of the minimum quantity of sulphur found effective against Wart Disease in the field to be in the form of thiosulphuric acid over a period of 10 days in order to account for its toxicity.

(5) Experiments of a preliminary nature carried out on sulphur-treated soil proved the formation of pentathionate in Rothamsted soil kept at 30° C., but not in Ormskirk soil kept at the same temperature, nor in either soil at the lower temperatures of 0° and 15° C.

(6) No definite evidence of the accumulation of appreciable quantities of thiosulphuric acid in the soil was obtained, but reasons are given why this negative evidence is by no means final.

(7) Chemical considerations and the work of others suggest that the pentathionate actually identified in the soil solution arose from thiosulphuric acid formed in an early stage of the oxidation of the sulphur.

(8) The explanation of the fungicidal action of sulphur towards Wart Disease in soil in terms of the formation of thiosulphuric acid is alone in harmony with the ascertained facts.

APPENDIX.

By R. P. HOBSON.

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After Mr Roach's departure from this laboratory, I continued for a short time the experiments started by him on the decomposition of sulphur in the soil and am taking this opportunity, offered to me by him, of presenting certain results which while of interest do not merit separate publication.

Incubations of sulphur and soil gave results which agree in general with Roach's findings. Sulphate was found to be the main product of the reaction and the only intermediate compounds whose presence could be established with any certainty were the polythionates. In one experiment carried out at 30° C. the amount of polythionates lay between 2 and 7 per cent. of the sulphur added (0·1 gm. per 100 gm. soil) for a period of 30 days. During this time the amount of soluble sulphur rose from 44 to 58 per cent. of the added sulphur. At temperatures of

0° C. and 15° C. polythionates were present only in traces, less than 1 per cent. of the sulphur added.

On one occasion a water extract of sulphur-treated soil gave a blue colour with acidified potassium iodide solution containing starch, which suggested the presence of persulphate. The extract, however, gave no blue colour with an alcoholic benzidine solution, a test which is sensitive to one part of persulphate per million. An extract of untreated soil with dilute sulphuric acid released iodine in the same way, probably through dissolving a metallic oxidising constituent, as aqueous extracts gave negative results. The sulphur-treated soil was alkaline (pH 8·3), but it is reasonable to suppose that local acidity in the region of the sulphur particles brought into solution the oxidising agent. There can be no doubt that persulphate was absent.

In order to test the stability of thiosulphate in soil, sodium thiosulphate was added to Rothamsted soil, in amount equivalent to 0·1 gm. sulphur per 100 gm. soil. After 5 days' percolation was carried out and the percolate analysed 5 per cent. of the sulphur was found in the form of sulphate, 84 per cent. as polythionates and only 11 per cent. as thiosulphate. Part of the last may have been present as sulphite which was not estimated separately. The disappearance of the thiosulphate was not due to the instability of thiosulphuric acid in acid solution, as the soil was slightly alkaline in reaction. It seems probable that such a powerful reducing agent cannot exist in the presence of material as labile as soil without undergoing oxidation.

In view of the rapid disappearance of added thiosulphate it is not surprising that thiosulphate could not be detected in sulphur-treated soil. As polythionates were the main transformation product of the added thiosulphate, it seems not unlikely that the small amounts of polythionates found in sulphur-treated soil originated from thiosulphate.

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(*Received October 3rd*, 1929.)

STUDIES IN THE PHYSICAL PROPERTIES OF SOIL.

V. THE HYSTERESIS EFFECT IN CAPILLARY PROPERTIES, AND THE MODES OF MOISTURE DISTRIBUTION ASSOCIATED THEREWITH.

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(With Six Text-figures.)

Introduction.

In two former papers (1, 2) the writer has dealt with the capillary properties of moist soil from both a theoretical and experimental standpoint. The first paper was followed by R. A. Fisher (3) with the presentation of certain emendations and criticisms, together with an elaborate treatment of a special part of the ideal case. Some of these criticisms were incidentally examined in my second paper in the course of a more extended treatment supported by fresh experimental results. Fisher returns to these points in a recent communication (4), and, among other arguments, makes the claim that a new theorem regarding stress must be taken as leading to a final rejection of certain of my views. It appears that this theorem is an elaboration of my first conception, and, while it gives precision where my own statement was crude, it is, nevertheless, in essential agreement with those first descriptions. In the present paper it is hoped to resolve the differences of opinion by the introduction of a fresh consideration which throws former arguments into a better perspective by showing where their basis was incomplete. Briefly, the new work has shown that two distinct cases must be considered, one for rising moisture and one for falling moisture. Confusion has been introduced and unfounded criticisms offered by the tacit assumption that moisture changes would form a reversible series, so that arguments applied to the features of one case have been used to exclude the possibility of the other. The changes in capillary suction which accompany cycles of change in moisture are shown in the experimental part of this paper to move round hysteresis loops rather than to and fro on a reversible curve. Neither theoretical discussions nor experimental studies have hitherto sufficiently emphasised this important distinction, and it constitutes a valuable standpoint for further work.

As an introduction the following simple illustration may be given of the essential basis upon which the hysteresis effect rests. Consider a vertical capillary tube having regular variations in its bore (radius r), dipping into a liquid by which it is wetted (surface tension T). In Fig. 1 let the sinuous line represent the characteristic of this tube, showing for any height above the free water surface the value of $2T/r$, which is the pressure deficiency shown by a liquid-air meniscus in the tube at the given place. The straight line OS represents the reduction in hydrostatic pressure with height for a liquid column in the tube. The points of intersection, R, X and S, where the abscissae of the two curves are equal, give the positions at which a meniscus can be in equilibrium with the column of liquid beneath it. The position X is unstable however. A slight displacement of the meniscus above X causes its pressure deficiency to outbalance the pull of the liquid column, and it moves up to position S. Thus an addition of water results in a rise of pressure deficiency. Similarly a displacement in the opposite direction causes a fall of the meniscus to R. Such *per saltum* movements have great importance in the analogous case of movement of the water boundary in the soil, when the moisture content is raised or lowered. R represents the position taken up for capillary rise of the column, while S is the position reached by a falling or draining column. By raising or lowering the tube the extreme values of the two heights will correspond to the extreme values in the fluctuation of bore.

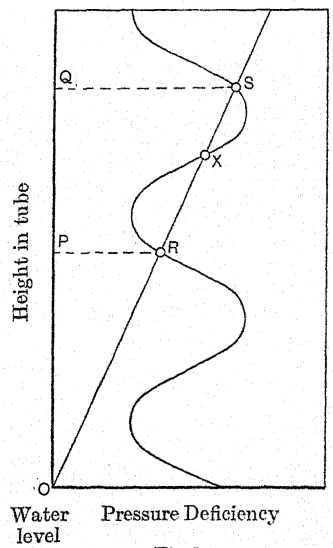


Fig 1.

Inasmuch as the pore space of soil has a cellular character, or a variable cross section, its capillary properties partake of the nature of those of the tube described. Except for limited ranges where reversible conditions may hold, the mode of moisture distribution in soil does not give reversible conditions but leads to two main values of capillary pull. The case of falling moisture tends to be governed by a higher value of pressure deficiency as determined by the narrower section of the pores, while conditions of wetting or increasing moisture tend to be governed by a lower value depending upon the wider sections of the pores. In other words, it is found that a granular system, of which soil is typical, will in

general offer a greater capillary pull against the extraction of water from its pores than it can engender when absorbing water into them. The distinction was clearly made in a former paper (2) when comparing capillary rise with drainage, but the full implications were not then developed.

THEORETICAL.

The theory of the subject has dealt with an assemblage of uniform spheres as the ideal soil. Two regular types of packing have been discussed, cubical and hexagonal, of which the latter will suffice for the recapitulated considerations which follow. The condition which determines the mode of distribution of any given amount of soil moisture is that the curvature at all points of the air-water interface shall be the same. This curvature is conveniently expressed in terms of the pressure deficiency or suction to which it gives rise in the soil moisture, and the natural unit for its measurement is the ratio T/r (T = surface tension, r = radius of particles). It is assumed that the effects of gravity may be neglected and that the angle of contact between liquid surface and solid particle is zero.

The nomenclature of Versluys (5) will be adopted for the types of distribution which arise. The lowest moisture range gives rise to isolated discs of water around the points of contact between particles, which is called the *pendular* case. This is the case which has proved amenable to full mathematical treatment. Its limit is reached when the discs are large enough to come into contact at their edges, which happens for a water content corresponding to 24 per cent. saturation of the pore space. But over a large part of that range of moisture the pendular form of distribution is not unique. For all higher moisture values the water must form a continuous body. Here two general types of distribution are distinguishable. In one, the *funicular* case, the discs of the pendular case become joined by fusion at their points of nearest approach, forming a meshwork with air still occupying the wider spaces of the pores. In the other, the *capillary* case, the water boundary which is considered encloses a saturated region of soil.

The cellular character of the pore space in the ideal soil has already been described in detail (2). It will be sufficient for the present purpose to recall that the dimensions are such that a hemispherical meniscus at the widest section (cuboidal cell) has a pressure deficiency of 6·9 T/r while one at the narrowest section (channel joining two cells) has the value 12·9 T/r. For the pendular case very small discs have a very high pressure deficiency which falls as the discs grow in size, reaching the lower limit of 4·5 T/r at the point where they are large enough for coalescence to

begin. This value is relatively low because of the anticlastic form of the water surface in this case. It may be likened to the deeply hollowed rim of a motor wheel as it is prepared to take the pneumatic tyre.

Changes of moisture within the pendular range may be made reversibly and with entirely symmetrical distribution through equilibrium with a soil atmosphere of changing relative humidity. For the other types of distribution with a continuous film, equilibrium is adjusted by direct flow of water, and changes are best considered as film movements or changes in curvature following direct increments or decrements of water. In the case of any soil air becoming trapped the means of attaining equilibrium becomes solution of air in the soil water. The distribution is not always symmetrical for higher moistures.

The total surface area of the interface between the soil moisture and soil air plainly passes through a maximum somewhere between zero moisture and saturation. On one side of this maximum the water surface increases with moisture, but on the other side it decreases with rising moisture. This leads one at the outset to doubt whether the entire range of changes could be conducted as a smooth reversible series of stable states.

It will be best to consider first in more detail the stable film forms which can exist, as determined by the geometry of the pore space, before proceeding to discuss the manner in which they may be associated for any given moisture content. The most simple case is the saturated or capillary one represented in Fig. 2 in a purely diagrammatic fashion. At (*a*) the fully saturated case is shown with a plane air-water boundary. For a very small water decrement the film is drawn into the surface pores of the soil as in (*b*). These pores have waist-like constrictions and open into wider cells beyond. The cells are of two kinds, the larger type cuboidal and the other tetrahedral. As the meniscii in the external pores advance inwards under the extraction of moisture from the soil, the pressure deficiency rises until the narrowest point is reached, when it has a value in the neighbourhood of 12 T/r. This might be called the liminal case of saturation. At some pore which is minutely wider than others the meniscus next passes the unstable point and expands abruptly into the cell beyond ((*c*) in Fig. 2). The displaced water redistributes itself with a slight general fall in the pressure deficiency. The shape of the film in the evacuated cell is made up of meniscii, one at each corner (except the one of entry), ready to penetrate farther into the soil by a similar leap. These meniscii are joined round the cheeks of the enclosing particles by films which are really broken-up portions of the pendular discs. This is the

funicular type of distribution. For further decrements in moisture the process of evacuation extends cell by cell through the soil. During the extraction of each quantum of water represented by a cell's contents the pressure deficiency rises gradually by a small amount (for a series of reversible changes), and then falls suddenly again (irreversible change). By repetitions of this process a large portion of the water is lost at a pressure deficiency oscillating slightly about the value at which entry first took place (entry value 12 T/r). The change is a simple movement, in quantum jumps, of a boundary between a saturated region of the soil and one having a funicular distribution.

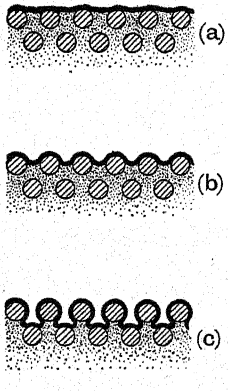


Fig. 2.

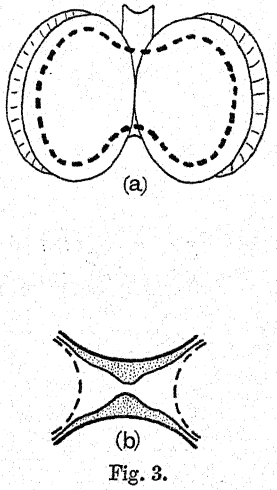


Fig. 3.

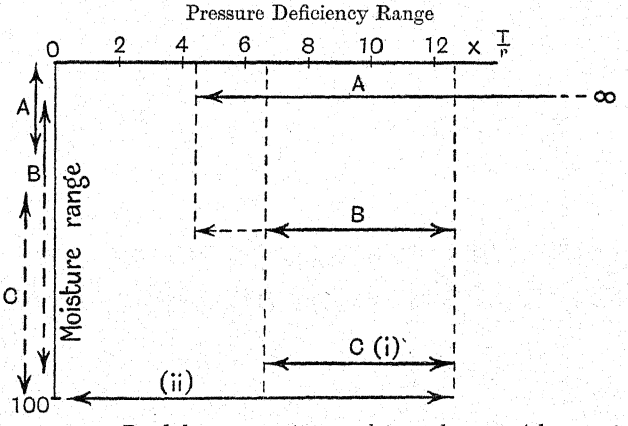
Consider now a reversal of the direction of moisture change at the stage represented by (*c*) Fig. 2. As the moisture increases and the pressure deficiency decreases the water film in each cell sags towards the centre, until in one cell (whichever is the smallest) the bubble detaches itself from the walls and collapses by evacuating the air through the external open pore. The point of instability for this is in the region of 6·9 T/r, the pressure deficiency of a bubble in the large type of cell. The entry remains "open" up to that point because the pressure would have to fall much lower to 4·5 T/r before closure takes place at a waist. The smaller type of cell will fill at a higher pressure deficiency, but they are always separated from one another by larger cells; hence they will fill in association with their larger neighbours in a manner which can be varied according to the suppositions made. Thus we see that a reversed movement of the boundary

round a saturated region takes place in a manner similar to its complementary case, that is by jumps, but at a lower pressure deficiency. Some confusion entered my former paper ((2), p. 273) by mistakenly identifying this value with the entry value, *i.e.* by not correctly separating the cases of rising and falling moisture.

A special feature relating to the funicular and pendular types of distribution is the existence of alternative positions which the film may take up in the waist-like connecting channel between two cells. This may be full of water with a meniscus at each end, or it may be open with an air-passage through. The receding film in the falling moisture case will open some passages, but leave others closed. Thus a cuboidal cell has eight corners, only two of which need to be opened by the transit of the water front, one for entry and one for exit. In Fig. 3 a diagrammatical presentation of a unit of the funicular case is given. At the limit of the pendular stage we have three "wheel-rim" surfaces set together at 120° with edges just in contact, forming an open hour-glass channel between them. In the full case each disc makes several such contacts with others round its periphery. At (*b*) is shown a diagram of a longitudinal section through the waist. As the discs close together by the addition of water a sudden closure takes place and the new surface is indicated in broken line in both (*a*) and (*b*). This closure is approached at a value of pressure deficiency in the neighbourhood of 4·5 T/r. As we have seen, when the moisture falls, the passage is only opened again for a pressure deficiency equal to the entry value. Thus full merging of the discs takes place for rising moisture at 4·5 T/r or lower, while their breaking up for falling moisture takes place at 12 T/r, which will give rise to discs of comparatively small size. These therefore are the two limits of pressure deficiency between which there exists the alternative of a waist being either open or closed, although it will be shown that the case for closed waists has only a limited possibility below the value 6·9 T/r. With sufficient waists closed to ensure moisture continuity we have the funicular case, within which changes are reversible and run approximately parallel to the values already given for the pendular case by Fisher (3).

The above facts are condensed in Fig. 4, which shows the range of pressure deficiency for each type of distribution. It is plain from this that all the various forms could be in equilibrium together within certain limits of pressure deficiency. There is by no means a close association between a given moisture content and a certain distribution, nor is the distribution usually uniform throughout the soil. The moisture ranges have been put in to show how they overlap, although the value 24 per cent.

saturation for the limit of the pendular case is the only one yet accurately determined. The overlapping between the moisture ranges of A and B is a genuine alternative distribution, but that between B and C is shown in broken line because part of the moisture is distributed in one form and part in another according to the amount present. The possibility of a partial development of the funicular case at pressure deficiencies less than 6·9 T/r is also shown by a broken line. Various cases of distribution can be arrived at according to the sequence of changes leading up to them. Over the greater part of the moisture range two regions exist side by side, one saturated and the other having a low moisture. This is modified when



A, Low moisture range. Pendular case up to complete coalescence (closure of waists).
B, Intermediate moisture range. Funicular case (after closure of waists).
C, High moisture range. Capillary case: (i) local saturation only; (ii) complete saturation.

Fig. 4. Scheme of various modes of moisture distribution and the ranges they cover.

a non-uniform soil is being considered. In an irregular medium the pore sizes vary, and the changes discussed tend to follow in sequence through groups of pores graded according to size. At any stage cells smaller than a given size will tend to contain water and cells larger than this to contain air.

One outstanding point remains, namely, to reconcile the value 4·5 T/r at the end of the pendular stage with the subsequent value 6·9 T/r when the funicular stage is giving place to saturation. In my earliest remarks (1), p. 531) the view was taken that the end of the pendular stage would be an unstable case followed by a rise in suction. It is plain that the point is approached at which further addition of water leads to decrease in surface area. For first the waists will close and then the cells. The

mechanism of the transition is not difficult to follow. At the end of the pendular stage the film shape in a unit cell can be imagined as a cuboidal (or tetrahedral) bubble rounded at the edges and drawn out at each corner to a cone-like extension, where it joins the neighbouring units in the hour-glass waists already depicted. These cones supply the outward tension which stabilises the natural inward tendency of the bubble to collapse. Closure of a waist corresponds to the collapse of the cone, and disappearance therefore of the compensating tension. This process will ultimately upset the stability of the bubble, which collapses, filling the cell with water and starting a region of saturation. To determine how many corners could be closed before instability is reached for each kind of cell would provide an interesting subsidiary problem. It would also illustrate the different ways in which rising moisture could be carried out by suitable choice of the order in which the waists are closed. Former arguments (2) were complicated unnecessarily by supposing that air would be trapped, but the closing of the waists in sequence could be conducted so as to avoid this. The important conclusion, which seems to the writer unavoidable, is that at some point the pressure deficiency will rise to 6·9 T/r for the completion of the range. The system is brought to a certain point at a pressure deficiency of 4·5 T/r (or rather lower) for which as many waists have been closed as is compatible with stability. Then the next closure of a waist is a trigger action which causes first one cell and then the next to close (compare a row of skittles). In this way the saturated zone extends, and a new equilibrium is not arrived at until enough water has been withdrawn from the other regions of the soil to raise the pressure deficiency to 6·9 T/r. The rise depends upon this general redistribution. The new case has a greatly reduced surface area, which meets difficulties raised from energy considerations. The readjustment gives the normal case for rising moisture over the higher range. Any alternative mechanism by which the last stages could be conducted at a pressure deficiency as low as 4·5 T/r would have to depend upon limiting the closure at the waists, if this would confine the unstable region to the zone in contact with the saturated water front. The point is rather an academic one, and only derives importance from the contrast between the theoretical approach from the coalescence of pendular discs which starts with the waists open, and the practical approach for cycles of moisture change which finds most of the waists remain closed.

Fisher has thrown doubt on this rise in the pressure deficiency above 4·5 T/r. Originally his considerations applied to "the series of states of the soil-water system in equilibrium with successive values of relative

humidity" as affording "a definite basis of comparison over the whole range from absolute dryness up to saturation" (3), p. 495). Such a series simply excludes a rise in pressure deficiency (synonymous with a fall in relative humidity) by definition, and is not necessarily valid for the criticism of a series defined by rising moisture content and passing through points of film instability. The relative humidity series passes from the unstable point directly to the case of final saturation for its next equilibrium position. For such a limited series Fisher's contentions may readily be conceded, but it may be remarked that the series gives undue prominence to the pendular (calculated) case while omitting cases of much more practical importance.

The points mainly contested by Fisher have concerned the stress or cohesion developed in the ideal soil. In my first approach to the subject I had considered the case of a saturated soil mass entirely enveloped by an inward-pressing water film. This led to the assumption that the cohesion for this case would equal the pressure deficiency, and a value between 6·9 T/r and 12·9 T/r was assigned to it. Fisher elaborates this conception of cohesion as produced by the inward thrust of the external layer of particles in the following theorem. If, for any layer of particles, the dry areas be divided into two groups, one having an outward aspect relative to the soil mass and total projection A per unit area and the other having an inward aspect and projection α, then the pressure exerted inwards depends upon the excess of A over α and is written

$$\text{stress} = (p + 2T/r)\,(A - \alpha),$$

where p is the pressure deficiency in the moisture. Applying this formula there appears no essential conflict with previous conclusions. For the approximate first assumption for the saturated case we see that the term $2\,T/r$ was overlooked, while A was given the value unity; whence stress = p since α is zero. In the more exact treatment for the liminal case of saturation we have $p = 12\,T/r$, $A = 0{\cdot}75$, and $\alpha = 0$. This case gives the maximum value of the formula, about 10 T/r, so that my estimate of a value lying between 6·9 T/r and 12·9 T/r cannot be regarded as deceptive. Nor was it deceptive to associate maximum stress with saturation. It may be noted in passing that it was the experimental agreement between the values of stress and of pressure deficiency at saturation in a sample of silt which led to the reopening of the argument. Both experimental arrangements had given the falling moisture case. The indication now is that this agreement was closer than was justified by the simple theory. The value found was 6 T/r for both measurements, and it is seen that the two deviations of the rough treatment from the exact one (supposing

direct application of the ideal formula to the real case) just happen to cancel for such a value.

Tracing the changes in cohesion as the moisture is decreased it is plain that α suddenly assumes positive values at the region of entry, without much change in A or p, so that cohesion falls. It seems that now there are two regions of different stress; that having the funicular type of distribution and a stress $(p + 2\ T/r)\ (A - \alpha_1)$ given by the external layer of particles, while the saturated region has an additional $(p + 2\ T/r)\,\alpha_2$, for the layer at the saturated water front. Thus the saturated region remains under the maximum stress. When the direction of moisture change is reversed the stress will be smaller, but similar conditions will hold. As the water front extends and obliterates α by filling the cells, the saturated region so formed gains an increased stress. The possibility of stress increasing with moisture for the later range seems clearly established.

Fisher comes to entirely contrary conclusions as to the application of the above formula, considering that it "effectively disposes of the notion of static stress rising to a maximum at saturation." Such reasoning must obviously depend upon the moisture distribution and as this is not described the argument lacks in definiteness. Although the formula is so well adapted for the exploration of the more important high moisture stages, Fisher contents himself with showing that it verifies the value already established for the end of the pendular stage. No sufficient guidance is given for an estimate of A, p or α over the range in question, although a general consideration of their changes is shown to lead to the conclusion that, if stress increases with moisture, then α must decrease more rapidly than A. Regarding this key condition it is simply stated that it has very limited possibilities. It has been shown, however, to be possible for the case discussed above. It seems that the changes in mind are smooth and reversible ones. If such a series can be theoretically established it may conform to Fisher's conclusions. But it must be entirely different from the changes here described, and shown in the next section to be reproducible experimentally. Since the irreversible series fall into line with experimental observations, they must be regarded as the ones which are important for consideration in practical problems relating to soil. In both his papers it is clear that Fisher, while contributing much to precision, has been in error in casting doubt upon the idea that high stress and high suction can arise for a saturated condition in the practical case.

EXPERIMENTAL.

In order to give the whole subject better objective illustration some simple experiments will be described in which the cycle of moisture changes was investigated under controlled conditions of pressure deficiency. The measurements bearing most closely on the ideal case were made by means of paraffin oil and phosphorbronze bearing-balls of $\frac{3}{32}$ inch diameter. These are large enough to arrange individually in the correct packing, but small enough to give measurable capillary effects. Time was not available to arrange large numbers for examination in bulk, but measurements were obtained for the behaviour in single cells in the following manner. A lead tube was taken for one arm of a U-tube and the top was shaped to take a porous plug of exactly the dimensions to form a plane surface on which a basic layer of particles in hexagonal packing could be laid. These were held in position by turning the edge of the tube slightly in the same manner as in setting a gem. Further layers could then be built up. The U-tube was completed with rubber and an adjustable second arm. This was then filled with oil, and the behaviour of the oil film between the particles examined through a lens while adjustments of pressure deficiency were made by raising or lowering the second arm. All entries between particles except those in the upper layer could easily be closed by placing sand or glass particles of appropriate size against them. This was desirable for studying strictly local changes. Measurements of head were made with a travelling microscope, while the surface tension was checked in all cases by means of the capillary tube method and by du Noüy's apparatus.

Under saturated conditions and for increasing suction the oil film could easily be seen making its abrupt entry into the first layer of cells. This value of pressure deficiency was between 11·3 and 11·5 T/r. For the return by diminishing values of suction the filling of a cell was equally abrupt, and could be demonstrated to take place first in those of tetrahedral form. The pressure deficiency value for this return again substantiated the theoretical values already discussed. If the receding film for preparation were dragged far, so that the pendular mode was mainly induced, the larger cells filled for the return at the value 4·3 T/r. This verifies the value for the coalescence of the pendular discs and demonstrates the subsequent instability in the immediate closing of the cell. The attainment of the low value simply depended upon the inability of isolated discs to adjust themselves as the pressure deficiency decreased, so that the saturated front as it advanced affected only those discs in actual contact

with it, the remaining region still having a high pressure deficiency round 11. In that case part of the contour of a cell remains unaffected until collapse. If, however, a single cell was dealt with, round which the oil continuity was such as to allow the whole film to sag naturally as the pressure deficiency fell, the collapse was at the value 6·7 T/r. The agreement with theory is so close that these higher values can never again be called in question.

A series of measurements of greater practical importance was made upon the material known as glistening dew. This is a tinsel consisting of minute glass spheres of very considerable uniformity, and it may be taken as closely simulating the ideal soil in all respects except closeness and regularity of packing. Green and Ampt (6) have described it in their own studies of soil permeability. The mean particle radius of the sample used was 0·019 cm. The natural packing, which could be altered slightly by the degree of shaking, gave a value of pore space between 36 and 37 per cent. or half-way between the ideal cases of close and open packing. The material was held in a Büchner funnel over a carefully sealed filter paper. This formed the terminal of one arm of a U-tube whose other arm was formed by a burette with double-bored stopcock (Fig. 5). The whole apparatus was filled with liquid and could easily be manipulated to apply changing suction to the material in small steps by adjusting the height in the burette. The resulting increment or decrement of moisture at each step after equilibrium was reached was read off on the scale. Reversibility could be tested for each section of the curves so obtained. The datum level for pressure was taken at the surface of the glistening dew. The whole mass had a depth of 2 cm. (and diameter of 9 cm.) and the results are therefore affected by this difference in gravitational head between the top and bottom of the material.

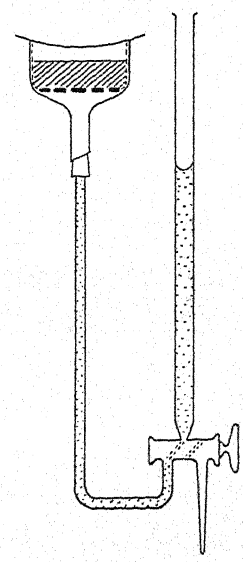


Fig. 5.

The irregular packing gives rise to a variable pore size, which is in the main wider than that for the ideal case and thus gives lower values of pressure deficiency. From the manner in which the water-air interface penetrates for falling moisture, it is apparent that the entry will be effected *last* into those places where the closest packing exists, that is, to such places as reproduce the ideal case. Therefore the ideal value for

entry may be expected to be shown at the end of the funicular stage. Conversely, for *rising* moisture, closure will take place *first* at the same places, and the ideal value will correspond to that point at which closure (and the consequent onset of irreversibility) can first be demonstrated.

A complete series of curves is shown in Fig. 6 with arrows to indicate the direction of the moisture change. Where the curve represents changes which are reversible it is drawn in double line. The nature of the experiment, of course, limits the findings to the case in which film continuity exists. The convenient origin for plotting was taken as 100 per cent. saturation and zero pressure deficiency. A glance at the figure shows the marked distinction between the falling and rising moisture cases, and the predominance of irreversible conditions. It is clear that there is no unique association between particular values of pressure and moisture. The material can be brought to the state represented by any point within the hysteresis loop by suitable cycles or raising and falling moisture. Over those stages where the curve is nearly vertical the moisture is not uniformly distributed throughout. As in the ideal case, a region of low moisture exists in equilibrium with a saturated one, and moisture changes go on by encroachment of one region on the other by movements of the boundary film. The existence of a gravitational gradient of pressure in the glistening dew determines that this boundary movement keeps to a horizontal plane.

Drying conditions usually predominate over wetting whether in the laboratory or field, so that the falling moisture curve *OABCDE* is the one of main interest. It is the same as that previously obtained by means of the manometer and porous pot (2). From *O* to *A* the pressure deficiency rises with only a small moisture movement confined to the rugosities of the surface layer. The change has already been depicted in Fig. 2, and is accompanied by a change in appearance from shiny to matt. At *A* the entry value for open packing is reached, and the first penetration of the film through the surface layer of particles takes place at isolated points. These points of penetration are plainly visible. As entry to a cell is effected the α dry areas (of the theoretical treatment) are formed inside, which greatly increases the internal reflection of the glass particles concerned. Entry is therefore accompanied by a sudden scintillation of the particles at the particular spot. General entry does not take place until the average value 6·1 is reached, indicated by the sharp bend in the curve at *B*. About 70 per cent. of the water is thereafter lost at this value. The portion of the curve from *B* to *C* would be quite vertical if corrected for the depth of the glistening dew (gravitational head).

An interesting indication of the effect of packing was obtained in one curve which showed a hump due to lower values of pressure deficiency obtaining after entry through the surface layer of particles. This was due to the extra close packing of the surface layer resulting from the greater freedom of the surface particles to roll about and adjust themselves.

Along the curve *CDE* the water loss is being supplied by evacuation of cells having smaller entries than the average and also by reduction of moisture under the funicular mode or film form. At *E* the funicular stage ends, the abrupt break being clearly indicated in the curve. No further water is removed by increases in suction. The value of moisture at *E* is 8 per cent. saturation, while the pressure deficiency should be that of the entry value of the ideal case as already indicated. Since the final break must occur at the junction between the glistening dew and the filter paper on which it rests, the value of 11·2 T/r on the curve at *E* requires to be corrected for this level. This gives the value 10·6 to compare with the value 12 given in the theoretical section. The suction value at *E* was the same for all repetitions of the curves, although there was some slight variation in other regards due to slight differences of packing obtained on different occasions. It is seen that the strict pendular case is confined under these conditions to the lower third of the moisture range which can be assigned to it in the theoretical treatment.

The curve for the rising moisture case is best followed by reversing at *D*, just before the break up of the film continuity. For the first part the returning curve runs parallel to the ideal curve for the pendular case (shown in broken line), since in the main the film forms correspond; that is, we have portions of rings joined together by those entries which have remained closed by reason of their tightness. This part of the curve is reversible. At *F* the curve bends away from this parallelism owing to the beginning of closure at the cells of tightest packing. This is also demonstrated by the fact that at *F* the curve begins to be irreversible. The pressure deficiency value at *F*, corrected for the depth of glistening dew, is 6·4, which is very near to the theoretical value for returning moisture. General closure for the average sized cell comes later at *G* for a pressure deficiency value of 4·1. It is interesting to notice the more angular nature of the change in the curve at *B* than at *G*. This is due to the more extensive air-water film in one case than in the other; so that in one case (*G*) cells of any one size throughout the mass have an almost equal opportunity to fill with water at their appropriate value of pressure deficiency, while in the other case (*B*) only those along the particular bounding water front have opportunity of evacuating water.

Saturation is practically complete at H. The unfilled volume of about 12 per cent. represented by KO is the air trapped in the process of closure, which tends to happen for abnormally big cells.

The experiments were repeated with paraffin oil instead of water, and exactly similar curves obtained. In this case the stable values were 5·9 T/r for falling "moisture" and 3·9 T/r for rising "moisture." The latter value is in perfect agreement with the mean value derived by Hackett (7) from measurements of the capillary rise of oil in sands.

A number of intermediate curves are shown illustrating the effect of reversal of the direction of moisture change at various stages. If the funicular stage is carried to its limit, the returning curve is via point L. Owing to the break up of film continuity and absence of the usual mechanism of adjustment, the form of the curve in such a case depends very much more than usually on the time allowed for equilibrium to be reached. The inside curve at L was obtained for very long periods (24 hours) between each reading.

The curve drawn in dotted line was obtained for a sample of sand with rounded grains. The much greater variation of grain size in this case gives rise to a finer pore space by adaptation in packing, and hence the suction values are higher. But the character of the two curves is unaltered.

As previously indicated ((2), pp. 275, 284) these curves may be viewed as showing the equilibrium distribution of moisture in a sand column above a free water table, if the suction values are first transformed into the heights which give the same gravity head. The right-hand curve is the case for drainage and the left-hand for capillary rise. The distinction needs great emphasis, since a great many studies of moisture distribution above a water table have been made without taking it into consideration. Indeed, it has generally been assumed that there is a single series of reversible changes.

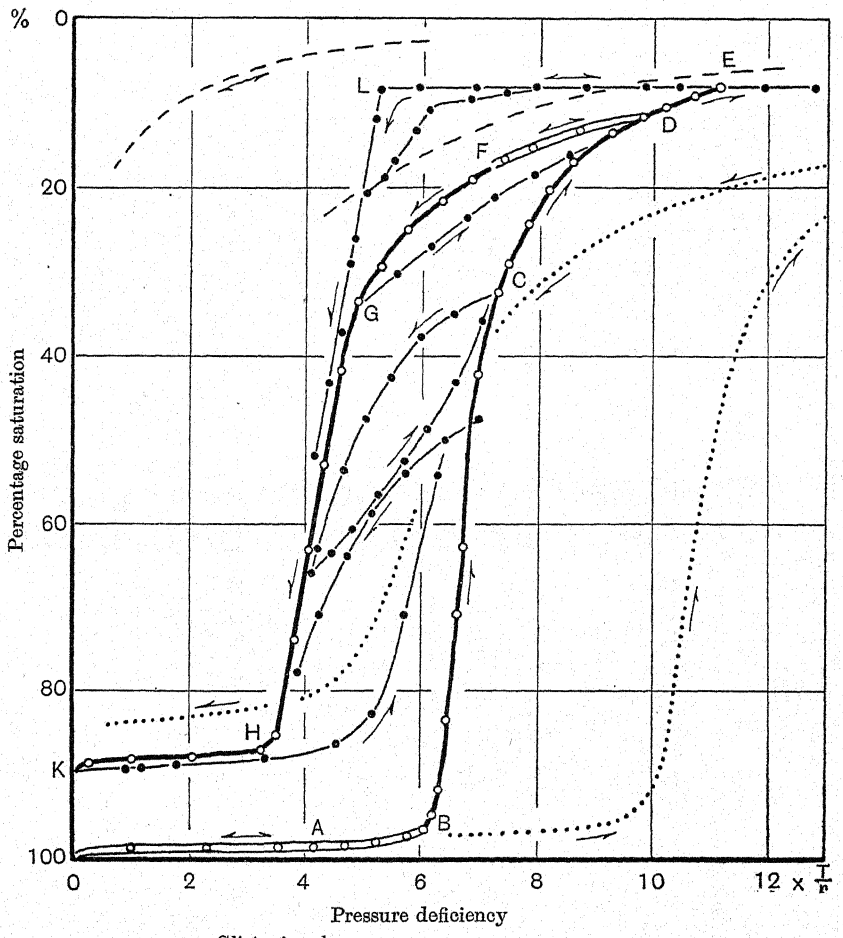
The work spent in removing moisture is $\int p\,dv$, or the area between the curve and the moisture axis. In the case of glistening dew and water the curve from O to E represented 42 c.c. of water removed, and the expenditure of $1{\cdot}02 \times 10^6$ ergs. This is of the same order as the surface energy of a water film equivalent in area to the particle surface. Both quantities reckoned per unit volume of soil vary as the reciprocal of r and therefore change in the same way with the texture of the soil. The area of any hysteresis loop is the energy wasted irreversibly.

Since writing the above an important contribution has been made to the subject by Hackett and Strettan (8). Their work elucidates the transition from close packing to the "common" packing naturally

assumed, so forming part of the essential bridge by which the theoretical case can be applied to real soils. Their treatment applies to the narrower pore sections which govern the falling moisture case. Their experiments are closely parallel to those reported here. Using steel balls and paraffin oil, the entry value found for close packing was the same as my own, namely 11·4 T/r. The manner in which it falls off with the angle of packing is very clearly worked out. The value for glistening dew is a good deal lower than mine at 4·75 T/r, but the difference is entirely explicable on comparing the two methods of measurement. While my own gives the value for the main group among the pore sizes, that of Hackett and Strettan gives the value for the largest pores. They cause a "saturated water front" to descend by drainage through a column of glistening dew resting upon wire gauze, and find the value for which it breaks through at the bottom. This takes place at the largest aperture available, as the authors clearly realise. Since, however, the stated aim is to infer the height of capillary *rise* in soil, the value 4·75 cannot be directly applied, and, compared with my direct measurement of rise, it must be regarded as a high rather than a low estimate for this. Actually the wire gauze itself forms the lowest stratum of apertures, and it would form with the bottom layer of particles a series of pores of different shapes to those which characterise the mass. Although an error due to such a cause would hardly escape the experimenter's attention, especially as the film movements were visible, yet the point is one requiring mention. In my own arrangement the boundary was the extreme case of such minute pores (of a filter paper) as to form a fixed barrier for the range of values being explored. In the other arrangement the supporting gauze must go to the opposite extreme and provide free passage through apertures larger than those which are required to be measured.

As regards stress between particles, certain observations can be made on the way in which this affects the friction between them. The glistening dew undergoes a most noticeable increase in firmness or rigidity while the suction is rising to the entry value, that is, between O and B in Fig. 6—very much greater than any subsequent change. A measurement was made of the depth of penetration of a loaded inverted cone into the glistening dew at different stages. The stress as so indicated was at stage B (liminal case) some ten or twelve times that at O (full flooding), which clearly proves the marked difference which can be produced by a very minute moisture change. The fall in stress subsequent to penetration of air was just indicated, but was too small to be definitely measured by the rough apparatus available.

The changes in appearance and in rigidity between *O* and *B* must be familiar to everyone in the behaviour of sea-shore sand under the foot when walking near a receding tide. The conditions then exactly reproduce



—— = Glistening dew
..... = Sand
- - - - = Theoretical, pendular stage, close and open packing.

Fig. 6.

those in the above experiments. The sand is saturated and under a small pressure deficiency. The additional property which comes into play is the Osborne Reynolds effect, namely, the anomalous dilatation of a closely packed granular medium when subjected to an external force. Pressure such as is given by the foot causes an expansion in packing and an increase

of void volume in the neighbourhood. This produces conditions equivalent to a movement along the curve *OB* in the direction of *B*, that is, to an increase in suction and in rigidity accompanied by a more matt appearance of the surface. If the pressure is maintained, a flow of water takes place toward the compressed region until the hydrostatic pressure is equalised. The imprint then left on removing the foot glistens (floods) for a moment, since the excess of water is released as the former packing is resumed. If the surface of the glistening dew be pressed by the finger at a similar stage in the above experimental arrangement, the Osborne Reynolds effect is demonstrated by a *fall* in the water level in the burette, showing the increase in pore space and in suction.

The criticisms raised by Fisher (4) can now be seen in their tr perspective, and the matter may be closed with the following few remar The calculation of stress or of work for the pendular case in the ideal s I cannot regard as having direct application to my measurements mad upon silt, since I have stated my disbelief that this distribution was eve actually attained ((1), p. 535). The experimental cohesion curve given, showing a maximum at saturation and a falling curve thereafter, plainly has the values and follows the course to be expected from the descriptions of this paper, bearing in mind that in the experiment the material was always prepared under drying conditions, so giving the falling moisture series.

Independent evidence of the increase of cohesion with moisture is afforded in the recent work of De Witt and Brown (9). These workers employed a much more accurate technique for determining the breaking strength of moulding sand. In the case where water was the only bond they report rising strength with rising moisture, while a rough calculation of particle size based upon the screening figures reduces the highest strength reported to the value 6 T/r, which was the value obtained by the writer.

Fisher's reference ((4), p. 410) to my demonstration of the micro-oscillations in the pressure deficiency associated with the quantum movements entirely confuses their significance. So far from being a series of oscillations after rupture of a film before a new mechanical equilibrium is established, they correspond individually to the invasion of a cell. My description showed that the rising half of each oscillation represents a reversible series of equilibrium values by which the limiting value of pressure deficiency is approached. A sudden fall completes the oscillation as entry to a cell is effected. The free oscillations consequent on this last adjustment are the ones referred to by Fisher, but they are

so highly damped as to escape attention in the apparatus which was described.

Fisher also expresses some confusion as to the point on the experimental curves which was identified with the entry value. The point of inflexion mentioned by Fisher, while useful as indicating the entry value for the main group of pore size, was not explicitly referred to. By definition the entry value was associated with saturation, and a comparison between the real and the ideal curves was relied on to make it plain that the usual convention was followed by which a sharp bend in an experimental curve is treated as an approximation to the intersection of two straight lines. This should not cause surprise by its arbitrariness. Other arguments advanced as to angle of contact and looseness of packing would, of course, strengthen rather than weaken the evidence already afforded that pressure deficiencies at high moistures could exceed 4·5 T/r.

SUMMARY.

A recapitulation of the main features of the moisture distribution in an ideal soil is given in order to emphasise a point previously neglected, namely, that the changes are not in the main strictly reversible, but fall into two series corresponding to the two directions of moisture change. The cellular nature of the soil pore space imposes a quantum character on the moisture changes over a great part of the higher moisture range. The individual cell does not fill or empty by smooth reversible changes but shows two unstable stages at which filling or emptying is completed at a bound. For falling moisture the suction level is that at which a meniscus can invade a cell through one of its narrow entries, which gives a value in the neighbourhood of 12 T/r for close packing. For rising moisture the suction level is that for which water returns to the cell by the collapse of a bubble in it, giving a lower value in the neighbourhood of 6·9 T/r. A still lower value of 4·5 T/r can be reached for a particular type of distribution confined to the lower moisture range.

All the above suction values are closely verified by measurements made with bronze balls and paraffin oil. A detailed exploration of the case for glistening dew and water has been made, which verifies the theory while illustrating more nearly the behaviour of an irregular soil. The two values which rule the two halves of the "hysteresis loop" for this case of natural or common packing are 6·0 T/r for falling moisture and 4·0 T/r for rising.

The new considerations resolve the differences of opinion raised by

R. A. Fisher. It appears that the author has throughout supported a case which belongs correctly to the falling moisture series. Fisher considered a very limited series for the rising moisture case and a hypothetical reversible series, and has been in error in so far as his treatment was regarded as exclusive.

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(*Received September 16th*, 1929.)

THE INFLUENCE OF A LOW AND HIGH CALCIUM DIET ON THE DEVELOPMENT AND CHEMICAL COMPOSITION OF THE SKELETON IN SWINE.

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The influence of a low and high calcium diet on the protein and mineral metabolism of pregnant sows, as well as on the appetite and digestibility of the food, was recently communicated in this *Journal* (1, 2). In view of the physiological effects described and the results obtained from balance experiments, it was considered that it would be of interest to compare the development and chemical composition of the skeleton in the two groups of animals, and to determine to what extent the composition of the bones had suffered under prolonged calcium deficiency.

Most of the work done on the chemical composition of the skeleton has been carried out from the point of view of studying the effects of "deficient diets" on the incidence of rickets. As a result of the discovery of vitamin D and the potency of cod-liver oil as a source of the anti-rachitic factor, several papers (3, 4) were published on this subject in 1925–26. It is generally agreed that in rickets the bones are mainly characterised by an excessive amount of organic matter and a low ash content. Low calcium rickets, however, differs from the ordinary type in that it cannot be demonstrated by X-ray, since there is no marked widening of the zone of uncalcified cartilage. On adding cod-liver oil to the diet the percentage of ash in the bones is distinctly increased, but when either calcium or phosphorus is deficient the ash content is still considerably below the normal. The ratio of lime to phosphate has been found to be practically the same for all bones, both normal and rachitic.

It is well known that a large percentage of winter-born pigs in this country suffer from rickets. This is attributed to the fact that in winter, the animals, being mostly indoors, suffer from lack of sunshine or vitamin D. It will, therefore, be interesting to find whether on a ration composed mainly of cereal grains, low in lime content, the addition of cod-liver oil safeguards the animals against rickets or whether the addition of lime is also necessary. In the ration used in this experiment all the factors with the exception of lime have been corrected.

Since full details of the experiment have already been published (1, 2), it will be sufficient here to give only a brief summary. It is important to note that the experimental animals were confined to concrete floors and were in the second or third generation of calcium deficiency, having been on their respective diets from weaning-time. The animals on the low-lime ration showed obvious signs of malnutrition, being periodically "off their feet" with their legs bending inwards and with a dirty scurvy appearance. This occurred in spite of the fact that they had a liberal supply of cod-liver oil incorporated in their food. The lime intake of the two groups naturally varied according to their live weight, but for the fully matured animals, while the normal sows received daily about 26–27 gm. CaO, the calcium-deficient animals only received approximately 1·7 gm. CaO. On this intake the normal sows retained a daily average of 6·4 gm. CaO, while the calcium-deficient animals were at lime equilibrium.

Method of obtaining bones for analysis.

The animals, after being slaughtered and opened, were allowed to chill overnight. The carcase was then cut into two halves a little to the left of the vertebra and the bones removed, from the right side only, so that as little flesh as possible adhered to them. They were then taken immediately into the laboratory and scraped clean of all flesh. The bones were then divided into four different groups[1] and were placed in weighed dishes and dried on a hot plate to constant weight. This gave the weight of dry bone.

The dried bones were first passed through a bone grinder and afterwards through a meat machine until they were reduced to a fairly fine and uniform condition. A known weight was then placed in a large Soxhlet apparatus and extracted with dry ether, so as to get an approximate estimation of their fat content. The fat-free bone was then passed through a mill and finely pulverised.

It was necessary in the first place, however, to find how much phosphate of the nature of lecithin was removed from the bones during the extraction process. Robinson and Soames (3) state that by extraction with alcohol and ether only 0·2–0·3 per cent. of the total phosphate of the bone is removed. In this work, using pure dry ether, it was found that not more than 10 mgm. of P_2O_5 were removed when 100 gm. of bone were extracted, this being a very small error considering the amount of phosphate present.

[1] See Table I.

THE CHEMICAL COMPOSITION OF THE BONES OF NORMAL AND RACHITIC YOUNG PIGS.

During the course of this experiment, five pigs belonging to the calcium-deficient sows, when 30 weeks old, went completely "off their feet" and showed typical symptoms of rickets. Since they could not stand up to feed and were losing weight rapidly, they had to be slaughtered. The bones of three of these animals were removed for analysis. The average live weight of these three pigs was only 71 lb., which is extremely poor for pigs of this age. In order to compare these bones with those of healthy normal animals it was decided to slaughter three pigs belonging to the high-calcium group, these being selected so that their average

Table I. *Giving the weight of dry bone* and the chemical composition of the fat-free bone (dry matter) of normal and calcium-deficient rachitic pigs.*

Group ... I	II	III	IV
Astragalus	Femur	Pelvis	Thoracic
Calcaneum	Tibia and Fibula	Sacrum	Lumbar
Tarsals	Patella	Tail	Ribs (3)
Cannon			Vertebra
Sesamoids			
Pasterns			
Coronet			
Coffin			

Average weight of dry bone (the above bones from one side only).

Group ...	I	II	III	IV	Total
Normal	70 gm.	118 gm.	65 gm.	117 gm.	370 gm.
Low calcium	47	77	37	68	229
Difference in wt.	23	41	28	49	141

Chemical composition of the dry fat-free bone %.

Group ...	I	II	III	IV
(1) *Normal pigs.*				
Organic matter	42·98 %	40·63 %	45·08 %	44·14 %
Ash	57·02	59·37	54·92	55·86
CaO	30·36	32·12	29·83	30·16
P_2O_5	23·52	24·71	22·65	22·92
N	6·33	5·61	6·42	6·30
Ratio: CaO/P_2O_5	1·29	1·30	1·31	1·31
Ratio: ash/organic matter	1·32	1·46	1·21	1·26
(2) *Low calcium rachitic pigs.*				
Organic matter	55·27 %	53·98 %	61·19 %	56·34 %
Ash	44·73	46·02	38·81	43·66
CaO	24·08	24·63	20·32	22·89
P_2O_5	18·72	18·94	16·30	18·09
N	7·62	7·38	8·71	8·10
Ratio: CaO/P_2O_5	1·28	1·30	1·25	1·26
Ratio: ash/organic matter	0·81	0·85	0·63	0·77

* It is important to note that the weights given throughout this paper refer to the particular bones mentioned and not to the whole skeleton.

weight was as nearly as possible that of the lime-deficient animals, their average weight being actually 86 lb. These pigs, however, were 10 weeks younger than the others. The average weight of dry bone and the chemical composition of the dry fat-extracted bones are given in Table I.

The poor development of the skeleton in the calcium-deficient pigs, although 10 weeks older than the normal pigs, is demonstrated by the fact that the total weight of the dry bones in the latter is 38 per cent. higher than in the former. This difference, as will be shown later, would have been still more pronounced if pigs of the same age had been chosen. Furthermore, the chemical analysis of the fat-free bone indicates a distinct lack of calcification in the case of the pigs suffering from rickets although both groups received cod-liver oil. The pronounced difference in ash content, which is over 12 per cent. higher in the normal compared with the rachitic bone, together with the high nitrogen and organic-matter content of the latter, all point to the fact that the calcified tissue in the rachitic bone must have been extremely thin. This is also in accordance with the observation made when the bones were scraped; those of the rachitic pigs being extremely soft and porous. Since there is reason to believe that rations similar in composition to that fed in this experiment to the calcium-deficient pigs—which contains approximately 88 per cent. of barley meal and maize meal in equal proportions—is commonly fed to young pigs in this country, the importance of adding a source of lime to the food, especially in winter, is clearly demonstrated.

The distinct difference in the ash content of the bones of the two groups is naturally reflected in the percentage of lime and phosphate present. It is noteworthy, however, that the ratio of lime to phosphate is approximately the same, both in the different groups of bones as well as in the normal and rachitic bone. This has been commented upon by Howland (4) and his co-workers, who state: "One is impressed with the constancy of the Ca : P ratio in the different bones of the same individual as well as in the bones of different individuals both rachitic and non-rachitic." The results clearly demonstrate that although vitamin D may be abundant, yet calcification is greatly retarded if the lime content of the food is at the low level of this lime-deficient ration. These calcium-deficient pigs of 71 lb. live weight were receiving about 0·5 gm. CaO per day, while the normal animals weighing 86 lb. would receive approximately 15 gm. CaO.

In view of the above results it will be of interest to investigate how far calcification had proceeded in the skeletons of the calcium-deficient animals when a year old compared with normal pigs 5 months old. For

this purpose, two calcium-deficient gilts were slaughtered when 12 and 13 months old respectively. The weight and chemical composition of the bones of these two pigs are given in Table II.

Table II. *Showing the weight of dry bone and the chemical composition of the bones of two calcium-deficient gilts when about* 12 *months old.*

Group ...	I	II	III	IV
(1) *Gilt* 64 S 7 (13 months old).				
Wt. of dry bones	197·00 gm.	428·00 gm.	254·00 gm.	390·00 gm.
Wt. of fat in bones %	32·50	45·60	32·60	25·00
Wt. of fat-free bones	133·0	232·8	171·2	292·5
	% composition of fat-free dry bone.			
Organic matter	41·28 %	40·23 %	43·89 %	44·91 %
Ash	58·72	59·77	56·11	55·09
CaO	31·92	33·02	30·85	31·86
P_2O_5	23·97	25·01	23·37	23·96
K_2O	0·096	0·160	0·190	0·204
Na_2O	1·080	0·999	0·950	0·925
N	6·134	5·735	6·479	6·566
Ratio: CaO/P_2O_5	1·33	1·32	1·32	1·33
(2) *Gilt* 15 P 2 (rachitic 12 months old).				
Wt. of dry bones	130·8 gm.	285·1 gm.	169·03 gm.	183·21 gm.
Wt. of fat in bones %	35·18	39·61	37·75	24·46
Wt. of fat-free bones	84·78	172·17	105·20	138·39
	% composition of fat-free dry bone.			
Organic matter	55·50 %	55·04 %	58·77 %	57·95 %
Ash	44·50	44·96	41·23	42·05
CaO	24·00	24·77	22·76	22·96
P_2O_5	18·46	19·20	17·72	18·37
K_2O	0·139	0·189	0·251	0·260
Na_2O	0·871	0·901	0·794	0·765
N	6·775	6·575	7·044	7·577
Ratio: CaO/P_2O_5	1·30	1·29	1·28	1·25

In spite of the fact that these two gilts were on the same ration from weaning-time, it will be observed that there is a striking difference in the weight and chemical composition of their skeletons. This is in entire agreement with their life-histories. The records show that 15 P 2 when 22 weeks old weighed 33 lb. and when slaughtered a year old her weight then was only 182 lb. When slaughtered, she was very lame and could hardly stand on her legs, having been suffering from rickets from April to November. Gilt 64 S 7, although she only weighed 21·7 lb. when 16 weeks old, seems to have made better progress and only suffered from mild rickets, her weight being 240 lb. when slaughtered at 13 months old.

A comparison of the results given in Table II with those already given in Table I brings out the interesting fact that the chemical composition of the fat-free bones of gilt 64 S 7 (13 months old) shows a striking similarity to that of the normal young pigs (5 months old), while the

bones of the rachitic pigs ($7\frac{1}{2}$ months old) resemble closely in composition those of 15 P 2 (12 months old). This indicates that while 15 P 2 was suffering from rickets, although the development of the osteoid tissue continued, yet there was no thickening of the calcified shaft, the ratio of ash to organic matter remaining fairly constant and exceptionally low. The calcified tissue in the bones of 64 S 7 was distinctly thicker, but the results show that calcification had already arrived at this stage in the normal pigs when only 5 months old or 8 months younger.

That calcification of the bones continues well into maturity is shown by the results given in Table III. The bones of Group I only are given in the table, since these are sufficient for demonstrating this point.

Table III. *Showing the increase in the calcification of the bones with maturity in the normal pigs.*

		7 S 1	8 N 2
Age ...	5 mths	1 yr 10 mths	2 yrs 9 mths
Wt. of dry bone	70 gm.	229 gm.	239 gm.
	% composition of fat-free dry bones.		
Organic matter	42·98 %	35·97 %	33·95 %
Ash	57·02	64·03	66·05
CaO	30·36	34·95	35·63
P_2O_5	23·52	26·57	27·25
N	6·330	5·131	4·998
Ratio: CaO/P_2O_5	1·29	1·31	1·31
Ratio: ash/organic matter	1·32	1·78	1·94

These figures clearly show that the shaft of calcification increases gradually in thickness, and that from 5 months old to maturity there is an increase of approximately 9 per cent. in the ash content even on a high calcium diet well supplied with cod-liver oil. It will, therefore, be interesting to find whether, in the case of the lime-deficient animals, calcification eventually becomes normal when the growth of the osteoid tissue has ceased, or whether both the rate and final degree of deposition of lime in the cartilage are depressed. The results obtained from the analysis of the bones of two fully mature animals from each group are given in Table IV.

If the weight of dry bones is taken as a basis of comparison, it will be observed that the calcium-deficient animals show a slight superiority over the normal sows. This, however, is due to the higher fat content of the bones of the former, and shows the importance of basing all conclusions on the fat-free bone. The weight of dry fat-free bone is 12 per cent. higher in the high calcium group compared with the calcium-deficient. Further, although calcification of the bones has distinctly

increased with maturity, yet in the calcium-deficient animals the percentage of ash is 2 to 4 per cent. lower, while the organic matter and nitrogen are correspondingly higher than in the normal group. The sodium and potassium content of the bones is slightly higher in the calcium-deficient group.

Table IV. *Showing the weight* and chemical composition of the bones of normal and calcium-deficient sows.*

Group ...	I	II	III	IV
(1) *Average results for the bones of* 7 S 1 *and* 8 N 2, *two normal sows.*				
Wt. of dry bones	283·00 gm.	727·00 gm.	443·00 gm.	852·00 gm.
Wt. of fat in bones %	17·08	26·80	28·65	14·75
Wt. of fat-free bones	235·00	532·00	316·00	726·00
	% composition of fat-free dry bones.			
Organic matter	34·96 %	32·87 %	36·76 %	37·52 %
Ash	65·04	67·13	63·24	62·48
CaO	35·29	36·14	34·39	33·80
P_2O_5	26·91	27·34	25·84	25·87
K_2O	0·1189	0·1012	0·1402	0·1962
Na_2O	0·8750	0·9310	0·8433	0·8174
N	5·064	4·694	5·297	5·377
Ratio: CaO/P_2O_5	1·31	1·32	1·33	1·30
Ratio: ash/organic matter	1·86	2·04	1·72	1·66
(2) *Average results for the bones of* 32 P 3 *and* 77 N 8, *two calcium-deficient sows.*				
Wt. of dry bones	295·00 gm.	727·00 gm.	479·00 gm.	952·00 gm.
Wt. of fat in bones %	28·5	38·7	48·3	27·6
Wt. of fat-free bones	211·00	446·00	248·00	689·00
	% composition of fat-free dry bones.			
Organic matter	37·28 %	36·46 %	40·53 %	41·21 %
Ash	62·72	63·54	59·47	58·79
CaO	34·33	34·59	32·90	32·51
P_2O_5	25·62	26·55	24·38	24·14
K_2O	0·1304	0·0990	0·1524	0·2259
Na_2O	0·9225	0·9215	0·8595	0·8939
N	5·346	5·189	5·629	6·063
Ratio: CaO/P_2O_5	1·33	1·30	1·34	1·43
Ratio: ash/organic matter	1·68	1·74	1·46	1·55

* These weights refer to the bones examined.

The significance of this difference in the chemical composition of the bones can be shown by computing the total amount of each constituent present in the bones of each group. The figures obtained are given in Table V.

The last column in Table V shows that in the bones of the right side only there is a difference in favour of the normal group of about 180 gm. tricalcic phosphate or in the whole animal of 360 gm. This is equivalent to an increase of 16 per cent. in the total tricalcic phosphate. A close

similarity is noted in the amount of soda and potash present in both the normal and calcium-deficient bones.

Table V. *Showing the total yield of the various inorganic constituents in the bones of the two groups of sows.*

Group ...	I	II	III	IV	Total
(1) *Normal sows.*					
Ash	152·8 gm.	357·1 gm.	199·8 gm.	453·6 gm.	1163·3 gm.
Lime (CaO)	82·9	192·3	108·7	245·4	629·3
P_2O_5	63·2	145·4	81·7	187·8	478·1
K_2O	0·28	0·54	0·44	1·42	2·68
Na_2O	2·06	4·95	2·66	5·93	15·60
(2) *Calcium-deficient sows.*					
Ash	132·3 gm.	283·4 gm.	147·5 gm.	405·1 gm.	968·3 gm.
Lime (CaO)	72·4	154·3	81·6	224·0	532·3
P_2O_5	54·1	118·4	60·5	166·3	399·3
K_2O	0·27	0·44	0·38	1·56	2·65
Na_2O	1·95	4·11	2·13	6·16	14·35

In order to find what effect lactation had on the composition of the skeleton, normal sow 10 S 2 was killed immediately after her litter of 12 young pigs had been weaned at 7 weeks old. The weight and chemical composition of the bones examined are given in Table VI.

Table VI.

Group ...	I	II	III	IV
Organic matter	36·35 %	35·99 %	36·52 %	37·62 %
Ash	63·65	64·01	63·48	62·38
Lime	34·69	35·22	33·92	33·10
P_2O_5	26·37	26·51	25·45	24·75
K_2O	0·113	0·124	0·162	0·220
Na_2O	0·918	0·917	0·884	0·860
N	4·979	4·788	5·320	5·453
Wt. of fat-free bones	212·00 gm.	482·00 gm.	318·00 gm.	589·00 gm.

The results show no appreciable difference in the chemical composition of the bones of,10 S 2 after lactation compared with the results given for normal sows in Table IV. The total weight of CaO in the bones of the former was 546 gm., while in the latter the amount calculated was 629 gm., the Ca-deficient sows having 532 gm. CaO. This shows that the calcium carbonate supplied in the ration of the normal sows was sufficient to protect the bones during heavy lactation.

Summary and Conclusions.

(1) The skeletons of young pigs on a ration composed mainly of cereal grains but with the addition of cod-liver oil show a very distinct lack of calcification, the percentage of ash being about 12 per cent. lower than in the bones of normal animals.

(2) Calcification of the bones proceeds up to maturity. A difference of about 360 gm. tricalcic phosphate was found between the same mature bones of normal and calcium-deficient animals.

(3) The ratio of lime to phosphate is almost the same in the different bones of the same individual as well as in the normal and rachitic bones of swine. The main characteristic of the bones in low-calcium rickets is a low-ash content, but the composition of the ash is normal.

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(*Received November 19th*, 1929.)

THE PROTEIN CONTENT OF GRASS, CHIEFLY MEADOW FOXTAIL (*ALOPECURUS PRATENSIS*), AS INFLUENCED BY FREQUENCY OF CUTTING.

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(With One Text-figure.)

The results of the first year's work (1927) in this investigation have already appeared[1], but in order that the significance of the second season's data may be the more readily apparent, the plan of the experiment may be outlined and a summary of the more outstanding results given.

Four plots (*A, B, C, D*) were staked out in a meadow of thick, uniform herbage, the predominating grass being meadow foxtail. Plot *A* was cut weekly, *B* fortnightly, *C* every third week, and *D* as hay with aftermath. The first cutting was made May 19 and the last September 29. The soil was a fairly rich, moderately heavy clay loam and no fertiliser was used.

Summaries for the several plots as to protein content of the grass, and yields of dry matter and protein per acre for the season, may be given as follows:

Protein and fibre content and yield of dry matter and protein, 1927.

Plot	No. of cuttings	Average protein (dry matter basis) (%)	Average fibre (dry matter basis) (%)	Yields per acre	
				Dry matter (lb.)	Protein (lb.)
A, cut weekly	10	21·20	19·38	2918	439
B, cut fortnightly	8	18·60	20·50	3344	466
C, cut every third week ...	5	17·17	22·06	4304	571
D, cut for hay, with aftermath	2	10·16	28·65	5311	520

Second season's results, 1928.

Immediately after the final cutting of the first season (September 29, 1927) an application of a complete fertiliser was made to all four plots. This consisted of ammonium sulphate 50 lb., superphosphate 350 lb. and muriate of potash 100 lb. per acre. Early in the spring of 1928 and before

[1] *J. Agric. Sci.* (1928), **18**, 411–20.

active growth, the plots were dressed with nitrate of soda at the rate of 100 lb. per acre. The plan of the experiment, as to frequency of cutting, etc., was that of the previous season.

The precipitation between the first and last cutting—May 11 and October 26—was 27·7 inches, an unusually heavy rainfall. There were several very heavy rains, and no week between the above dates passed without at least a shower.

The scheduled dates of cutting were interfered with in a number of cases by rain, it being necessary for a satisfactory carrying on of the work that the plots should be reasonably dry when cut. The season as a whole was undoubtedly favourable for the scheme, though at certain periods there was too much rain for optimum results.

FREQUENCY OF CUTTING AS AFFECTING PERCENTAGES OF MOISTURE, PROTEIN AND FIBRE.

The herbage for 1928 on all four plots shows a decidedly higher percentage of moisture than that of the previous season. This, in part, may be accounted for by the absence of all dead grass from the preceding year (present to some extent in the earlier cuttings of 1927), and in part possibly by the heavy rainfall of the 1928 season, which kept the growth fresh and green. There was no period of drought during which the grass might partially dry out.

Table I. *Moisture content of grasses as cut*, 1928.

	Plot *A* 16 cuttings (%)	Plot *B* 9 cuttings (%)	Plot *C* 8 cuttings (%)	Plot *D* 3 cuttings (%)
Maximum ...	82·61	80·38	83·60	74·99
Minimum ...	72·92	71·15	68·70	64·41
Average ...	78·66	78·68	77·16	68·81

Little difference is to be noticed between plots *A*, *B* and *C* in respect to moisture content—there was no steady decline with the lengthening of the periods between cuttings as noted in the previous season—but plot *D*, cut for hay, with two aftermaths, as might be expected, is markedly lower.

PROTEIN.

The protein percentages of all four plots, and more especially those of *A* and *B*, are very much higher than those of 1927. In respect to plots *A* and *B*, this is undoubtedly due to the rapid encroachment by white Dutch clover; by the end of July, *A* was carpeted with this plant over the greater part of its area, while in *B* this clover had spread over more

than one-third of the plot. At this period in 1927 there was only an occasional plant of this clover on these plots. The growth and spread of this legume to such a degree as to practically drive out the grasses must be attributed to the conditions—mainly exposure to light-incident to this scheme (see Fig. 1).

On plot *C* clover began to show in the herbage during September and October—and this is reflected in the higher protein recorded

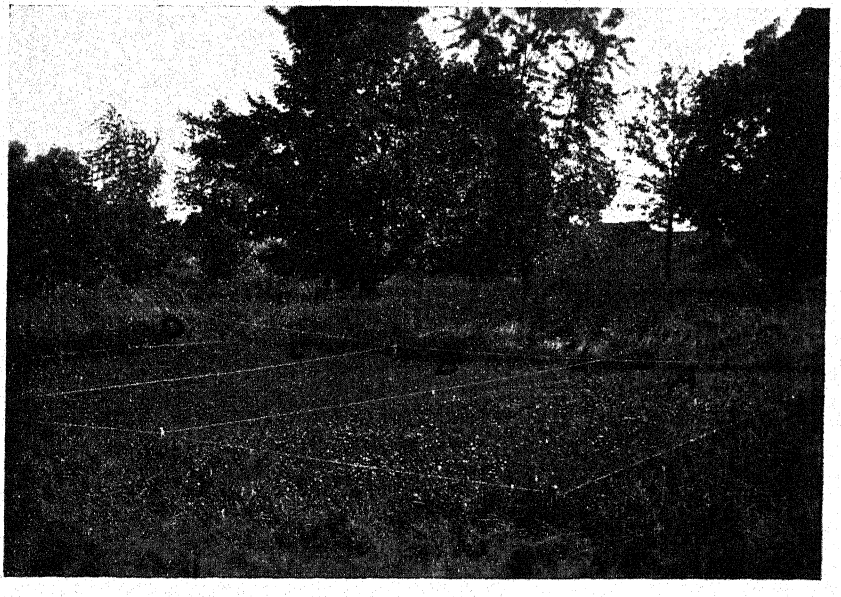


Fig. 1. Close-grazing plots.
Arboretum, Central Experimental Farm, Ottawa. (Showing white Dutch clover in bloom on *A*.) June 26, 1928.

during the latter part of the season. At the time of the last cutting the clover had become firmly established over possibly one-fifth of the plot.

No white Dutch clover was observed on plot *D* at any time during the season, but a few plants of red clover were to be noticed.

Another factor contributing to these higher protein percentages and affecting the herbage of all four plots is the fertiliser applications; the two dressings of nitrogen (sulphate of ammonia in the autumn of 1927 and nitrate of soda in the spring of 1928) might assuredly be credited with some share in raising the protein content of the herbage.

Table II. *Protein* (*dry matter basis*), 1928.

Dates of cutting	Plot *A* 16 cuttings (%)	Plot *B* 9 cuttings (%)	Plot *C* 8 cuttings (%)	Plot *D* 3 cuttings (%)
May 11	29·95	—	—	—
18	28·45	26·04	18·52	—
26	28·75	—	—	—
June 1	25·24	17·80	—	—
8	28·26	—	—	—
15	26·69	21·37	14·52	—
July 3	27·65	23·80	—	11·96
6	—	—	20·99	—
13	28·39	—	—	—
20	29·38	21·99	—	—
27	32·60	—	19·44	—
Aug. 10	28·34	20·82	—	—
17	31·30	—	22·17	—
27	31·26	23·65	—	11·61
Sept. 7	28·29	—	24·90	—
22	31·05	22·39	—	—
28	—	—	25·72	—
Oct. 26	27·56	24·96	19·64	16·34

The foregoing data are summarised in Table III.

Table III. *Protein* (*dry matter basis*), 1928.

(Maximum, minimum and average data.)

	Plot *A* 16 cuttings (%)	Plot *B* 9 cuttings (%)	Plot *C* 8 cuttings (%)	Plot *D* 3 cuttings (%)
Maximum ...	32·60	26·04	25·72	16·34
Minimum ...	25·24	17·80	14·52	11·61
Average ...	28·95	22·54	20·74	13·30

In 1927 the increase in protein content from *D* to *A* was to be attributed, very largely, if not solely, to an increase in the frequency of cutting, or, stated otherwise, to the fact that very young grass is richer in protein than that of more mature growth. The higher protein content of *A*, *B* and *C* in 1928, as compared with that of *D*, follows the operation of two factors: frequency of cutting and the development of clover—with the latter the more potent of the two.

FIBRE.

The results of 1927 showed very distinctly that frequency of cutting markedly affects the fibre content; the longer the period of growth the higher the percentage of fibre. From this, and the relation of protein content to frequency of cutting, it necessarily follows that high protein is associated with low fibre and *vice versa*.

Table IV. *Fibre* (*dry matter basis*), 1928.

Dates of cutting	Plot *A* cut weekly (%)	Plot *B* cut fortnightly (%)	Plot *C* cut every third week (%)	Plot *D* cut for hay with aftermath (%)
May 11	13·79	—	—	—
18	15·12	16·82	22·30	—
26	18·41	—	—	—
June 1	18·65	20·86	—	—
8	17·88	—	—	—
15	17·31	20·49	23·86	—
July 3	18·75	20·89	—	34·33
6	—	—	22·72	—
13	18·64	—	—	—
20	18·82	21·57	—	—
27	17·52	—	22·88	—
Aug. 10	15·54	20·24	—	—
17	16·02	—	20·76	—
27	16·83	20·22	—	28·69
Sept. 7	14·78	—	19·36	—
22	14·05	16·25	—	—
28	—	—	14·34	—
Oct. 26	12·39	12·65	11·96	17·54

The 1928 data support and confirm those of the preceding season in respect to the relationship of fibre to protein, and, further, show that the trend of fibre content as influenced by frequency of cutting—the younger the grass the lower the percentage of fibre—is maintained.

Table V. *Fibre* (*dry matter basis*), 1928.

(Maximum, minimum and average data.)

	Plot *A* 16 cuttings (%)	Plot *B* 9 cuttings (%)	Plot *C* 8 cuttings (%)	Plot *D* 3 cuttings (%)
Maximum ...	18·82	21·57	23·86	34·33
Minimum ...	12·39	12·65	11·96	17·54
Average ...	16·53	18·88	19·77	26·85

Table V presents in summarised form the fibre data of the season. The percentages for all four plots are decidedly lower than those of 1927. This is accounted for on *A*, *B* and *C* by the large proportion of leafy clover present and in the case of *D* by the two aftermaths of comparatively young grass.

To obtain data applicable to the present discussion as to the protein and fibre content of grass and clover, respectively, analysis was made of the herbage of Plot *A* of the cutting of July 3 as a whole and of its grass and clover separately.

Analysis of herbage of Plot A. *Cutting of July* 3, 1928 (*dry matter basis*).

	Herbage as cut (%)	Grass (%)	Clover (%)
Protein	27·65	23·40	30·70
Fibre	18·75	25·07	17·29

These figures show the influence on the herbage of clover, in raising the protein and lowering the fibre.

FREQUENCY OF CUTTING AS AFFECTING AMOUNTS OF DRY MATTER AND PROTEIN, PER ACRE.

Dry matter yields.

Table VI presents, in addition to weights of dry matter, the dates of cutting, data of precipitation and mean temperatures for the growth period preceding each cutting.

Table VI. *Weight of dry matter, lb. per acre*, 1928.

Date of cutting	Precipitation (inches)	Temperature (mean)	Plot *A* cut weekly (lb.)	Plot *B* cut every two weeks (lb.)	Plot *C* cut every three weeks (lb.)	Plot *D* cut for hay with aftermath (lb.)
May 11	—	54·7° F.	34·9	—	—	—
18	0·04	50·3	293·8	698·9	—	—
26	1·48	56·8	420·8	—	1249·6	—
June 1	0·64	53·7	245·3	968·4	—	—
8	0·45	54·2	196·9	—	—	—
15	0·37	60·2	190·6	351·6	976·0	—
July 3	5·48	64·2	550·5	423·5	—	6579·4
6	0·75	69·3	—	—	388·4	—
13	0·66	71·1	237·0	—	—	—
20	2·41	69·1	130·1	535·3	—	—
27	0·17	65·7	228·3	—	608·5	—
Aug. 10	3·45	63·9	484·9	875·6	—	—
17	0·25	69·3	139·7	—	404·6	—
27	1·23	66·0	143·9	333·5	—	2412·0
Sept. 7	0·43	66·5	146·1	—	260·0	—
22	3·19	56·2	145·1	284·3	—	—
28	0·81	44·1	—*	—*	114·0	—
Oct. 26	5·93	48·7	181·9	139·2	47·4	315·0

* Growth too short to allow of cutting.

As in 1927, the season of 1928 was characterised by a very heavy and well-distributed rainfall. The total precipitation between May 11 and October 26, the period between the first and last cuttings, was 27·7 inches —an amount greatly exceeding the normal, and no week passed within this period without rain. At certain periods during the season the intervals between the rains were so short that it was not possible to adhere

to the regular scheduled dates of cutting, it being found impracticable to satisfactorily harvest the plots immediately after a heavy rain. It is obvious that rainfall is a factor of prime importance to the success of a close-grazing scheme; a sufficiently ample and well-distributed precipitation greatly aids the rejuvenation of the grazed area—periods of severe drought result in very slow and sparse growth. The season of 1928 was on the whole undoubtedly very favourable to the scheme, though possibly at certain periods there was too much moisture for optimum growth.

The yields from all the plots in 1928 are greatly in excess of those of 1927, and this is to be attributed to the application of fertiliser, associated with a longer growing season and a favourable moisture supply.

The results in general show that yields, as in 1927, are closely related to the precipitation of the preceding growth period. The work of both seasons has shown that moisture, *i.e.* rainfall, is the factor of first importance towards recovery and vigorous growth. Further, in this connection, it was noted that as clover replaced grass, as more particularly in Plots *A* and *B*, recovery was quicker than in the preceding season, when grass was the predominating herbage.

Seasonal yields, dry matter, per acre.

	Plot *A* cut weekly (lb.)	Plot *B* cut fortnightly (lb.)	Plot *C* cut every third week (lb.)	Plot *D* cut for hay with aftermath (lb.)
1927 ...	2918	3344	4304	5311
1928 ...	3770	4610	4048	9306

Much larger yields were obtained from Plots *A*, *B* and *D* in 1928 than in 1927. This heavier herbage is attributable to a number of factors, chief among which are the increasing replacement of grass by clover, the influence of fertiliser applications, a thickening of the "stand" by cutting and a longer season in which the growth received no set-back from the periods of drought.

The heaviest yield was obtained, as in 1927, from Plot *D*. There was a very heavy growth, as cut for hay on July 3, with two good aftermaths —the last date of cutting, October 26, indicating an exceptionally long season of growth.

The positions of *B* and *C* in relation to each other are reversed to those held in 1927, the yield of *B* now exceeds that of *C*. Weather conditions prevented the regular fortnightly harvesting of *B* to such a degree that only one more cutting was made on it than on *C*, and this removes from consideration the factor of "interval" or frequency of cutting. Plot *C*

is the only one of the series which gave a lighter yield in 1928 than in 1927. This plot, due to its topography, suffered from flooding and gullying, with a destruction of grass over a part of its area. This naturally reduced its yield.

The dry matter yield of *A*, as in 1927, is the lowest in the series.

The results of 1928, outstandingly those of *A* but also notably those of *B* and *C*, have furnished confirmatory evidence of the depressing action of frequent cutting on yield. The data of both seasons have conclusively shown that the plot least frequently cut (*D*) has given the highest yield of dry matter.

Protein yields.

The protein yields in 1928 for all four plots greatly exceed those of 1927. Factors affecting all the plots of the series and favourably influencing yields were a good growing season and the fertiliser applications made in the fall of 1927 and the spring of 1928.

Seasonal yields: protein, per acre, 1927 *and* 1928.

	Plot *A* cut weekly (lb.)	Plot *B* cut fortnightly (lb.)	Plot *C* cut every third week (lb.)	Plot *D* cut for hay with aftermath (lb.)
1927 ...	439·0	466·0	571·0	520·0
1928 ...	1078·0	1008·0	766·0	1119·0

Plot *D* furnished the heaviest weight of protein, but this yield, however, is closely followed by those of Plots *A* and *B*. Owing to the length of season there were two aftermaths from this plot and these furnished growth of higher protein content and digestibility than grass cut for hay. But since the protein of the hay (cut July 3) constitutes seven-tenths of the total protein yield of *D*, and is of lower digestibility than the protein of *A* and *B*, it may be safely concluded that, although *D* furnished slightly the highest weight of protein, this protein did not equal in nutritive value that of the more frequently cut plots.

The protein yield of *C* in 1927 was higher than that of *B*; the reverse is true in 1928. This exchange of position was due in part to destruction of herbage on *C* from flooding, and in part to the spread of white Dutch clover on *B*. It was seen in discussing dry matter yields that *C*, in 1928, similarly fell behind *B*. Thus it would appear justifiable to consider Plot *C* in the second season, by reason of its injury, as temporarily removed from the scheme.

The protein yield of *B* is practically equal to that of *A*. This, in a large measure, is to be attributed to the fact that towards the close of the season

the herbage of this plot consisted of probably 70 per cent. of white Dutch clover, thus approximating in botanical composition that of *A*.

Plot *A* in this second season was essentially clover, and this accounts for its very high protein yield associated with its comparatively low dry matter yield. It is of interest to note that in the short course of two seasons the character of herbage and its nutritive value could, by the adoption of a close-grazing scheme, be so profoundly changed.

Summary.

From a study of the data of two season's work the following observations may be made:

Frequency of grazing with a view to the successful operation of the scheme will depend on a number of factors, chief among which is the character of the herbage and its growth. Recovery and vigour of growth are primarily dependent on rainfall and secondarily on the fertility of the soil, which may be increased by fertiliser applications. Rainfall, its amount and distribution, has in this investigation shown itself the most potent of all influences in the recovery of the plots. The results in general of 1928 indicate that the application of fertilisers, in which nitrogen predominates, greatly assisted in the maintenance of vigorous growth.

The protein content of the herbage increases with the shortening of the period between cuttings, due in the first season solely to the fact that the protein content of the grass falls off with age, but in the second and subsequent seasons, in a very large measure, to the incursion of white Dutch clover.

A consideration of protein yields per acre in relation to frequency of cutting reveals that in the first season, when the herbage is essentially grass, the shortening of the period between cuttings tends to reduce the protein yield; in the second season, when clover replaces grass, roughly in proportion to the shortening of the growing period, the higher protein content of the legume tends to counteract the depressing effect on yield of frequent cutting.

There is evidence to show that yield of dry matter per unit area decreases with frequency of cutting. In both seasons the largest yield of dry matter was obtained from the plot cut as hay with aftermath.

The close-grazing scheme, according to the plan here adopted, appears to be essentially one productive of herbage of high protein content and digestibility.

(*Received September 11th*, 1929.)

LATERITE AND LATERITIC SOIL IN SIERRA LEONE, II.

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(With Four Text-figures.)

In a previous communication (1) a short description was given of laterites and lateritic soils in Sierra Leone. As a result of an enquiry into definitions of the terms "laterite" and "lateritic" the writers proposed that, when these terms are applied to soil, those soils whose clay fraction had a silica/alumina ratio (molecular) of less than 1·33 per cent. should be called laterites, those soils where this ratio exceeded 1·33, but was less than 2·0, should be termed lateritic, while those soils whose clay fractions have a silica/alumina ratio greater than 2·0 should not be described by any term denoting an excess of aluminium. These definitions of laterites and lateritic soils will be used in this paper.

There are in Sierra Leone two forms of laterite, residual and detrital. Residual laterite (2) is that laterite which still occupies the place in which it was developed, while detrital laterite is that material which has been removed by erosion from the place in which it has been lateritised, or partially lateritised, and which has accumulated by deposition in another place. Any partially lateritised material thus deposited can, if the conditions be favourable, still undergo further lateritisation in its new surroundings.

The formation of laterite in the Sierra Leone Peninsula.

The formation of laterite in the western half of the Sierra Leone Peninsula has been carefully studied. This section of the Colony is composed of a mountainous mass of norite which together with narrow coastal fringe of Pleistocene sediments makes up this part of the Peninsula (3). The rocks forming the hills have been considerably weathered and much lateritised material has been formed *in situ*, and still remains *in situ*, in the hills. This is regarded as residual laterite or residual lateritic material. The hills are covered with a mass of vegetation of the ordinary West African "bush" type, while the soil except where it is stained by humus is generally of a reddish colour, although in places it is more yellow than red.

On the lower and flatter slopes of the hills is an accumulation of material which has been washed down from the higher slopes. This material, judging from present day conditions, originally consisted of lateritised and partially lateritised material, but further lateritisation of the deposited material has taken place and is undoubtedly still taking place, while the formation of concretions of laterite or lateritic boulders gradually goes on in this detrital deposit.

Much confusion exists over the terminology at present used and the term "lateritisation" is often employed to indicate the formation of the red vesicular rock so characteristic of lateritised material. This vesicular rock is often developed by the cementing together with iron of material which has previously lost most of its combined silica and is therefore already laterite. In this paper therefore the terms "lateritisation" and "formation of laterite" are used to indicate the processes of removal of silica from the original rock, whereby a residuum is left containing a high proportion of alumina as compared with silica. Whether this mass contains much or little iron, is cemented together in a hard mass or is in a soft crumbly condition, is immaterial, all substances with a low silica/alumina ratio formed by the weathering of rocks are regarded by us as laterite or lateritic, and the process leading to this condition is called lateritisation.

Residual laterite. On examination of the norite rocks of the Colony, it is found that where they are not exposed to the atmosphere they show a margin of soft yellowish white material speckled with dark brown. The unchanged norite passes abruptly into this yellowish white material, and analyses (Table I) of this light coloured substance, taken as near the rock as possible (without including undecomposed norite), show that it has reached a high stage of lateritisation. The original minerals of the norite have become entirely changed to a mass of yellowish flaky material, while the iron of the ferruginous minerals has become segregated into the cracks and cavities formerly occupied by the mineral, in the form of dark brown opaque compounds of iron. This is a condition previously found and described by Bauer (4) in the Seychelles.

Table I.

Sample no.	Description	SiO_2	Al_2O_3	Fe_2O_3	CaO	Loss on ignition	Silica/alumina ratio (mol.)
—	Norite rock	49·6	17·9	11·9	18·8	0·6	4·69
—	Layer near rock	7·3	41·5	23·2	Trace	26·6	0·30
2396	Layer 3 in. from rock	7·3	44·7	17·2	Trace	27·9	0·28
2392	Layer 6 in. from rock	9·3	50·8	12·8	Trace	26·1	0·31
—	Bauxite (3)	9·5	51·1	14·1	—	25·3	0·31

If the rocks are not disturbed or exposed to the atmosphere, the weathering or lateritisation continues, concentric layers of yellowish white laterite as described above being formed round the rock.

Analyses (Table I) show that these layers differ very little in composition as regards the proportions of silica and alumina present, but the behaviour of the iron is of interest. In the layer near the rock the iron is highest, and it decreases as the layers become farther removed from the original rock. Where this form of weathering has gone on for a long time, the norite rock has in places nearly disappeared and left behind it the lateritised material from which much iron has also been removed. This material is commonly known as bauxite, low grade deposits of which were found by Dixey (3) at Wilberforce: these bauxite deposits were of a similar composition to the layer taken six inches away from the norite rock (Table I).



Fig. 1. Showing vesicular laterite rock being formed in the fissures between norite rock; in between is the flaky whitish material.

In the hills of Sierra Leone there is much of this bauxitic material, although probably not of a sufficiently high grade or in sufficient quantities to be workable. It is often exposed when cuttings are made for roads, railways, etc.

It is not always that the iron is thus removed from the lateritised material leaving bauxite, this only happens when the conditions (presumably movements of water in the soil) are favourable for the removal of the iron. In other cases the iron, which in the soft yellowish white speckled material is not a binding agency, may undergo a change without necessarily being removed, and, by cementing together the material with which it is in contact, form the vesicular orange-red rock so characteristic of laterite. A case in point is shown in Fig. 1. In this photograph the original norite is shown split along definite lines. In the crevasses the soft yellowish white laterite is first formed with the iron distributed through it in dark brown compounds,

but subsequently this iron has undergone a change. No longer is it in dark brown coloured masses embedded in the yellowish material, it has become orange-red in colour, holes have formed in the matrix and an orange-red vesicular rock has been formed, the iron supplying the cementing agency; this, as its analysis (Table II) shows, is laterite rock.

Table II.

Sample no.	Description	SiO_2	Al_2O_3	Fe_2O_3	CaO	Loss on ignition	Silica/ alumina ratio
2534	Norite rock	44·3	26·9	19·2	8·6	0·2	2·8
2533	Intermediate products	28·3	28·7	21·6	Trace	20·9	1·66
2532	Laterite rock	17·9	26·01	39·7	Trace	14·6	1·17

All the samples in Table II were taken within a radius of six inches and were taken from material developed along the cracks of norite rock: there was therefore very little chance of any material, except possibly small quantities of iron in percolating water, being brought in from outside, and, in any case, the overlying rocks were thin and of the same nature.

The two samples quoted above are perhaps somewhat extreme cases of lateritisation, the one without removal of iron and the other with considerable removal of iron. In the majority of cases the iron is not so completely removed (leaving bauxite), nor does the vesicular rock always form so closely to the parent rock. What is frequently formed is something in between these two extremes, the bauxite is not so free from iron and instead of the vesicular rock a sort of loosely coherent scaly highly ferruginous mass is formed, the composition of which varies from that of the bauxite to that of the laterite rock. A cutting through such an area is shown in Fig. 2.

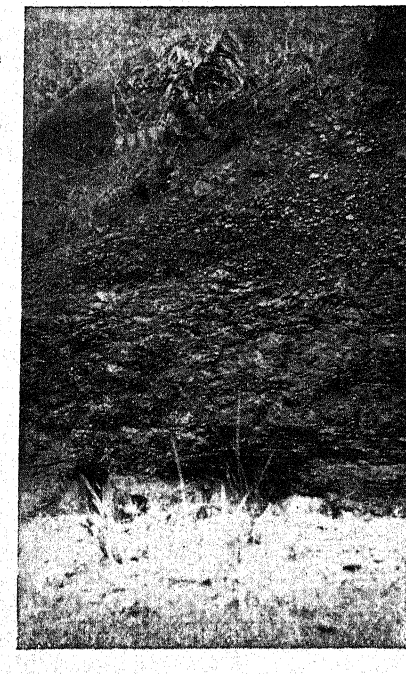


Fig. 2. Lateritisation of norite, showing formation of a loosely coherent scaly ferruginous mass.

The soil formed *in situ* from this norite formation varies with the degree of lateritisation of the rocks with which it is in contact. In colour the soil is yellowish to red and the

surface soil is stained a light or dark brown by humus. The soil is usually very stony, containing anything from 30 to 70 per cent. of particles greater than 2 mm. diameter. The "stones" are concretions of laterite having a low silica/alumina ratio (generally less than 1·0) and a high iron content (40 to 50 per cent. Fe_2O_3), in fact similar in composition to the vesicular laterite rock formed *in situ*.

Analyses of the various fractions of laterite soils have already been given by the writers(1), and it is obvious from the figures in Table I and those previously given that lateritisation is not the only agency at work in forming soils, since, if this were so, the soil would contain a much higher proportion of aluminium than is actually found. Disintegration goes on as well as lateritisation, but any fragments of rock split up by disintegrating agencies (*e.g.* rapid changes in temperature) are in turn subject to lateritisation. In addition to this, the iron present in the soil undergoes changes and acts as a cement, binding together particles of soil to each other and forming ever-growing concretions.

The silica/alumina ratio of clays from soils formed *in situ* as a result of lateritisation of norite vary from 1·05 to 1·69 so far as our experience goes, and in the clay fractions of these residual laterites we have never yet found a clay fraction with a lower silica/alumina ratio than 1·05. In order to try and get a "clay" with a lower silica/alumina ratio, we obtained some of the yellowish white flaky loosely coherent material from the margin of a norite rock, which had a silica/alumina ratio of 0·18, crushed it with a wooden rolling pin and shook with ammonia for several hours to deflocculate any clay particles present. The clay was separated by sedimentation and analysed; the silica/alumina ratio was 1·41. This was repeated with another sample of the same kind of material, the silica/alumina ratio of which was also 0·18; the separated clay fraction had a silica/alumina ratio 1·54. The analyses of the whole of the two flaky laterite deposits and the "clays" derived therefrom are given in Table III.

Table III.

Sample no.	Description	SiO_2	Al_2O_3	Fe_2O_3	Loss on ignition	Silica/alumina ratio
2512	Flaky yellowish white laterite	5·6	53·1	12·0	28·0	0·18
—	Clay from 2512	33·0	36·3	9·5	19·0	1·54
2513	Flaky yellowish white laterite	5·8	54·3	11·1	28·2	0·18
—	Clay from 2513	31·4	37·8	11·6	18·4	1·41

Detrital laterite. As a result of erosion deposits are formed on the flatter slopes and at the bottoms of the hills in Sierra Leone. There is little doubt that the material washed from a higher to a lower slope was

originally of much the same composition as the surface soil now is on the higher slopes of the hills, that is to say, it is a deposit composed of lateritised and partially lateritised fragments of norite. These fragments have undergone further lateritisation, and particles have been cemented together by iron, forming concretions. We have not yet met a case where the iron appears to be removed from detrital laterite, as happens during the formation of bauxite higher up in the hills.

The cementing agency of the iron tends to increase the proportions of the larger fractions in the soils, and these detrital laterite soils contain a high proportion of stones and gravel (about 50 to 80 per cent.). Frequently huge boulders are formed, while in extreme cases a laterite pan may be formed below the surface of the soil, only a film of soil being left above the pan of laterite rock. This film in extreme cases is only sufficiently thick to support certain grasses instead of the ordinary "bush" growth of the country. Fig. 3 shows a photograph of such an area, while Fig. 4 shows a cutting through an area where such a pan is in process of formation. In this latter photograph the growth of the vesicular laterite rock below the surface of the ground can be clearly seen.

The changes which take place during this process of cementation are of interest and can be studied by taking and analysing samples of the soil, the partially cemented material, the cemented material and the hard laterite pan, where these all occur within a short distance of each other. This has been done, and the samples reduced to powder in an end runner grinding mill; the powder was fused with fusion mixture and analysed with the results given in Table V.

The effects of the lateritising process going on in this detrital deposit are clearly shown. The silica is being removed while the iron and aluminium both remain behind, and it will be seen that the proportions of aluminium and iron with respect to each other remain the same throughout the series of samples examined. The silica/alumina ratio decreases during the formation of these concretions of laterite while the loss on ignition, chiefly water, slightly increases, due presumably to the increased proportion of aluminium hydroxide present.

Table IV.

Sample no.	Description	SiO_2	Al_2O_3	Fe_2O_3	Loss on ignition	Silica/ alumina ratio
2389	Rubbly soil	23·1	27·7	30·7	16·8	1·41
2390	Partially cemented material	17·5	27·1	35·7	17·5	1·10
2390	Selected lumps from 2390	9·5	32·9	37·7	18·8	0·49
2391	Firmly cemented material	10·0	33·1	38·1	17·3	0·51
2388	Rock from old laterite pan	3·6	35·2	38·8	20·4	0·17



Fig. 3. Showing an area of laterite pan. The vesicular laterite is seen outcropping in the left-hand bottom corner, while the film of soil on the rest of the area only supports grass life.

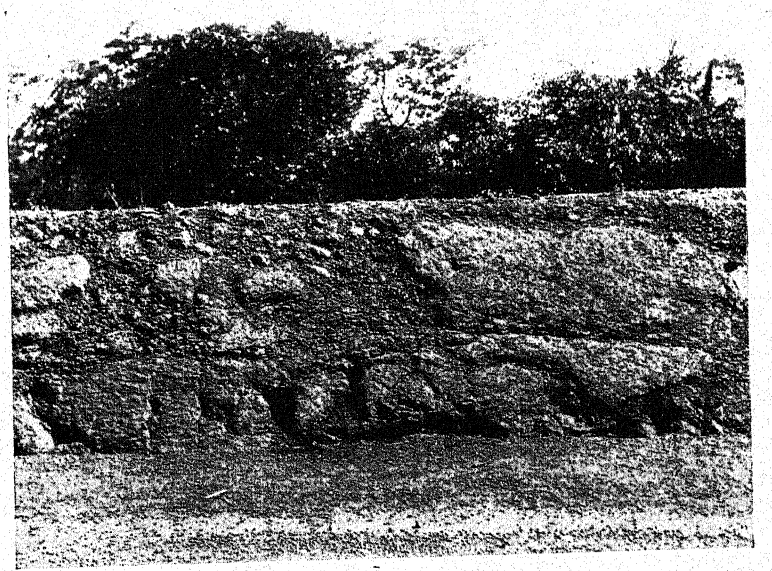


Fig. 4. Showing formation of laterite rock beneath the surface of the soil. Note that the "bush" is weak on this type of soil.

The soils of these detrital laterites are similar to the soils of the residual laterites, except that on the whole they have a rather lower silica/alumina ratio. Many of the soils formed *in situ* in the hills are of course not laterites since the silica/alumina ratios of the clay fraction often exceeds 1·33, but as the ratio is less than 2·0 the soils are lateritic. The detrital soils in the neighbourhood of Freetown, on the other hand, are usually laterites.

CHARACTERISTICS OF LATERITES AND LATERITIC SOILS IN SIERRA LEONE.

The soil formed from the norite hills in the Peninsula of Sierra Leone are of a red or yellowish red colour, although they may be stained by humus to a black or dark brown colour on the surface. In texture the soil is light and permeable and generally contains a high proportion of "stones" or "concretions," some of which may be of a very large size. The hydrogen-ion concentration is high, the *p*H value lying generally between 4·5 and 5·5, with the highest concentrations in the surface layers and a slight decrease in concentration with depth. The "lime requirement" varies between 0·15 and 0·45 per cent., being on the average about 0·3 in the first foot and gradually decreasing to about 0·2 in the fourth foot. The exchangeable calcium is low, generally less than 0·1 per cent. CaO (0·04 to 0·08 per cent. in those cases examined), and appears to be correlated to some extent with a degree of lateritisation of the soil, although not sufficient figures are yet available to say anything very definite about this.

The mineral content is low and the following figures were obtained by digesting samples of the "fine earth" portions of the soil with strong hydrochloric acid.

Table V.

Description of soil	K_2O	P_2O_5	Silica/alumina ratio of clay fraction
Gravelly laterite	0·02	0·15	0·77
Gravelly laterite	0·02	0·12	1·29
Gravelly laterite	0·02	0·07	1·32
Lateritic gravel	0·03	0·08	1·36
Lateritic gravel	0·04	0·14	1·49

The potash content of all these soils is very low and there appears to be some correlation between the amount of potash and the silica/alumina ratio, the less the ratio the lower the potash. This correlation is to be expected, as the weathering agents responsible for removing the silica would also remove the more soluble potash.

The phosphorus content on the other hand is not so strikingly low, possibly because any phosphorus in the soil may combine with the

free aluminium and iron present to form insoluble compounds. This seems to be borne out by an experiment with a number of lateritic soils. Twenty grams of soil were shaken with a solution of acid calcium phosphate containing 0·04 per cent. of P_2O_5 and left for five days. The suspension was filtered, the phosphate in the filtrate estimated, and from that the percentage of phosphorus removed by the soil was calculated (Table VI).

Table VI.

Soil	Percentage of P_2O_5 removed from solution	Silica/alumina ratio
2387	99·3	1·36
1105	98·2	1·66
2514	96·5	1·75
2505	97·7	1·89
2340	72·1	2·11

The results (Table VI) show that the soils are capable of removing large quantities of phosphorus from solution, while a comparison with the silica/alumina ratio suggests that the higher the proportion of free alumina the greater the proportion of phosphorus removed.

SUMMARY.

1. A study has been made of the formation of lateritic soils in Sierra Leone and the composition is given of some of the intermediate and final products formed.

2. The detrital soils in Sierra Leone appear on the whole to contain a higher proportion of alumina to silica than the residual soils.

3. The soils described are all reddish or yellowish red soils, light in texture and generally containing a high proportion of stones. They are acid in reaction and have a definite lime requirement. The mineral constituents are low, especially the potash and the exchangeable calcium.

4. Soluble phosphorus is readily removed from a solution by these soils.

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(*Received October 23rd*, 1929.)

VOLUME XX APRIL, 1930 PART 2

THE ANALYSIS OF THE LACTATION CURVE INTO MAXIMUM YIELD AND PERSISTENCY.

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(With Ten Text-figures.)

INTRODUCTION.

DURING recent years biometricians have eagerly explored the field opened up by the practice of milk recording, and in the comparatively short time that figures have been available much valuable information has been gained. In common with others this branch of science has had to pass through an empirical phase in its evolution, and by analogy we must expect this to be succeeded by a more analytical one—that is, the gross effects of various factors having been demonstrated and measured, the next enquiries will be as to their mode of action, in the hope of acquiring further knowledge of the underlying physiological processes. Although the results of such studies may lack immediate practical value they must always have a greater appeal to the scientist, since they involve definite analyses with a nearer approach to the fundamental, and it is clear that further advance must be halting and uncertain until this line has been pushed forward considerably. Nor can the biometrician regard his efforts with any complacency until he has probed his material to the bottom, and defined the physiological problems with all possible exactitude—only then can he feel that his responsibility has ended, and that their final solution may be left to the experimental biologist.

The standardised lactation yield is a direct measure, on a comparable basis, of the average rate of activity of the mammary gland over a long period, but it cannot be regarded as a single entity, for, apart from diurnal fluctuations, the rate of secretion displays definite trends throughout the milking period; consequently it is a complicated compound, the detailed investigation of which demands its division into its components and their separate study. As a general rule the yields of individual cows subsequent to parturition rise to a maximum in a few weeks, and then fall slowly away until secretion stops, so that the first and most obvious analysis of the curve will be into the two variables—(*a*) the height to which the yield rises, and (*b*) the rate at which it falls off from this

maximum. The study of the first presents no difficulties, and considerable work was done on it until it was realised that the maximum yield by itself was unsatisfactory as a measure of the cow's productivity, since the second property (persistence of secretion) also varied from cow to cow; it followed that both variables must be investigated together.

Persistency is rather difficult to study since it is by no means easy to find a good scale along which it can be measured. Gaines (5) has published the results of an investigation on these lines; his method was to fit an exponential curve to each lactation and study the two parameters in this, the first of which gave a theoretical initial rate of secretion immediately after calving (not attained in practice owing, in Gaines' view, to the slow exhaustion from the blood of an inhibitory hormone formed during pregnancy), and the second a direct measure of the fall as the lactation progressed. In many ways this method is ideal, but, apart from the labour involved in fitting the curves (which must necessarily limit the data), many irregular cases must occur in which the actual curve fitted depends to a considerable degree on the judgment of the fitter. Turner (15) has used the percentage fall in yield month by month and this method obviously presents great possibilities, since it enables the rate of fall to be studied at all stages of the milking period; as yet, however, he has confined himself to the average percentage for all months of the lactation.

There is another possibility; it has been said that the lactation yield is the result of two variables—the maximum yield and persistency—and it is most natural to regard it as the product of the two; consequently as a reasonable definition we have

$$\text{Persistency} = \frac{\text{Total yield in lactation}}{\text{Maximum yield}}.$$

This ratio is, perhaps, rather crude, but it should serve as a first step in the analysis of the lactation curve; it gives a definite figure for each case, and is clearly a direct measure of what is generally understood by persistency; it is true that it gives no detail as to the shape of the lactation curve (where, for instance, the maximum is reached) but, on the other hand, it loses none of its meaning whatever that shape may be.

In order to make the measure more readily intelligible it was decided to divide the above by the normal ratio; in this way it was reduced to a figure fluctuating about unity—all lactations giving a figure greater than 1·00 were more persistent than normal, and all giving less were below normal in this respect. Furthermore, with ordinary herds conditions vary considerably throughout the year, so that cows calving at different

seasons tend to show different degrees of persistency; accordingly it was decided to vary the divisor with the month in which the cow calved, so that the seasonal effect might be eliminated at the outset.

The data consisted of 3918 lactations of cows of various breeds recorded by the Norfolk Milk Recording Society; these data have been fairly fully described in the report of a statistical enquiry into the various factors affecting the total lactation yield (12). Table I shows, for each month of calving, the number of records, the mean total lactation yield, the mean maximum weekly yield, and the ratio between the last two.

Table I.

Month of calving	No. of lactations	Mean total lactation yield (lb.)	Mean maximum weekly yield (lb.)	Lactation yield / Maximum yield
January	381	7081	254·2	27·86
February	391	7218	269·1	26·82
March	332	6953	269·6	25·79
April	292	6993	285·8	24·47
May	232	6649	270·8	24·55
June	208	6389	254·0	25·15
July	287	6567	255·1	25·74
August	263	6522	244·1	26·72
September	312	6667	231·1	28·85
October	415	7099	241·7	29·37
November	406	7111	250·3	28·41
December	399	7375	260·3	28·33
Mean	—	6940	257·2	26·84

This table shows that persistency is largely affected by the season of calving; the ratios are all above the average for the months September to January and below for the others. These ratios were used as divisors, and the figure reached (which has already been used by the writer (11)) was called the shape figure (S.F.) of the lactation. Consequently we have

$$\text{Shape figure} = \frac{\text{Total lactation yield}}{\text{Maximum yield} \times R},$$

where R takes the value given in Table I according to the month of calving. As a matter of detail, a table was drawn up for each month showing the values of the Total/Maximum giving S.F.'s proceeding by 0·01 from 0·70 to 1·30; with the aid of these, S.F.'s could be calculated very rapidly.

It must be admitted that this is purely a "research" figure and not well adapted to practical use; it is regarded solely as a means to an end —as a measure of this rather elusive property of persistence, giving a definite value to it for each lactation, values greater than unity showing persistence above the normal and *vice versa*; with its aid an attempt has

been made to investigate the two main features of the lactation curve in the data mentioned above.

This study falls into three parts:

(1) The effect of various factors on the maximum and on persistency; seasonal influences having been eliminated there remained service period, age of the cow (in lactations) and the length of the preceding dry period, details of which were available; the study of the effects of these on maximum and persistency serves as an analysis of their gross effects on the lactation yield.

(2) From the present data corrections have already been calculated (12), enabling a record to be standardised in respect to the above-mentioned factors; nor are these by any means unique; numerous other workers have produced similar correction tables for various factors, and, in general, the agreement between them is good. But it is well known that in many individual cases they are disappointing in their application, though dependable in the long run, and one is naturally curious to know why with some cows they appear to be so inaccurate. It appears probable that greater precision could be obtained (were it worth the largely increased labour and difficulty in use) by the preparation of separate tables for particular groups of cows; in this respect the writer has tried dividing cows into groups on the basis of breed and lactation yield level, but neither of these held out much hope of success. The factors concerned may affect the yield differently at different stages of the lactation, so that it seems that their effects must vary somewhat from cow to cow according to the relative yield level at the stage at which their action is most marked—in other words it might well be that correction factors should be varied with the persistency of the cows to which they are applied. Consequently the data have been divided into two groups—high and low S.F.'s—and the variations caused by the four factors, month of calving, service period, age, and dry period, have been ascertained from each group. On the whole the differences between the groups were hardly as striking as had been anticipated, but some were discovered; these help to show how errors arise in correction, and demonstrate conclusively the futility of very precise correction factors for general use—it is clear that with these differences in being, curves which disregard them by averaging all levels of persistence together, cannot apply very closely to individual cows, and hence cannot give percentage corrections accurate to several decimal places.

(3) The relative importance of the maximum and of persistency in determining lactation yield; information has been sought by considering

the differences, with these two, between breeds, between herds, and between cows in the same herd (where the environment may be taken as approximately constant). The last provides a means of testing the relative constancy of maximum, persistency, and total lactation yield, throughout the cow's life.

PART I. THE EFFECTS OF SERVICE PERIOD (S.P.), AGE, AND DRY PERIOD ON MAXIMUM AND PERSISTENCY.

Service period.

The effect of pregnancy on the lactation curve has already been determined from these data (12); briefly, the yield is only slightly affected in the early part, but at mid-pregnancy a considerable drop occurs, and secretion thereafter slows down rapidly until the cow is dry; this state-

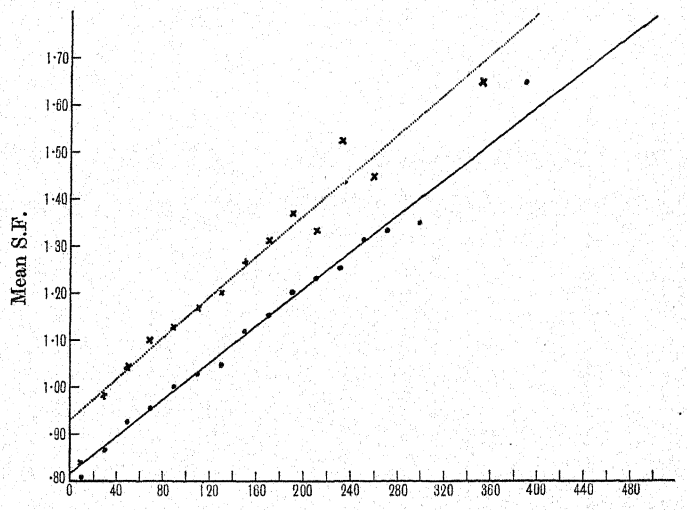


Fig. 1. The effect of the length of the S.P. on the S.F.
1st calvers ..... × × Others ———— . .

ment is supported by the work of Gavin (7), Turner (13) and Gaines and Davidson (6). Under these circumstances it is to be supposed that (except in a few cases of very early service) the maximum will not be appreciably affected by the length of the S.P., but the persistency will be largely influenced by it. Fig. 1 shows the relation existing in these records between S.F. and the length of the S.P.; since the effect of this variable on total lactation yield has already been found (12) to differ

slightly between 1st calvers and older cows, the figure shows these two groups separately; there were 879 lactations of 1st calvers and 3039 lactations of older cows.

The correlation tables from which this figure was obtained gave the following statistical constants:

	1st calvers	Others
r	$+0{\cdot}571 \pm 0{\cdot}015$	$+0{\cdot}530 \pm 0{\cdot}009$
η	0·582	0·542
$N(\eta^2 - r^2)$	11·146	39·082
$a-2$	14	18
P	0·674	$<0{\cdot}01$

These values of r are noticeably higher than between length of S.P. and total lactation yield, which were + 0·444 and + 0·407 respectively—a result to be expected since service affects lactation yield through its constituent persistency, with little or no effect on maximum.

The above values of r and η are slightly higher for 1st calvers than for the others, but the differences are not significant; $N(\eta^2 - r^2)$ tends to be distributed as χ^2 when n is taken as two less than the number of arrays (4), and it is seen that with 1st calvers the regression approximates closely to the linear, since the value of P derived from this is 0·674. With the others, however, P is less than 0·01, indicating that the regression is really curvilinear, but the figure shows that the straight line cuts through the points well, and that no means depart very far from it. As a matter of fact the means rise in an interesting manner—three "waves" can be detected; the vast majority of observations (93 per cent.) fall in the S.P. intervals below 140 days, and the means for these rise rapidly at first and then flatten out, apparently tending to a limit; those between 140 and 240 days show exactly the same thing, and there is evidence of yet another "wave" with the next three means; it seems certain that the average cow in the S.P. intervals below 140 days would not attain a higher S.F. than about 1·20, however long she was left empty, and, similarly, the next group appears to have an upper limit of about 1·40. The conclusion seems inevitable that we have here a definite "selection" for high persistency as we pass from short to long S.P.'s and this might arise in four ways:

(1) Persistent cows may be deliberately left empty longer than those whose yields fall away rapidly.

(2) There may be a tendency to dispose of non-persistent cows unless they are pregnant early in lactation—if a cow's yield has fallen to a low level before she is served the owner may well consider it uneconomic to keep her.

(3) Some farmers may make it a rule to serve their cows as soon as their yields have dropped below some definite rate.

(4) There may be a physiological relationship, for it is well known that fertility and milk secretion are closely related; Hammond(8), indeed, has produced evidence that some specific substance (or substances) is necessary for the functioning of both the mammary glands and the ovaries, and that if the former are using it rapidly the activities of the latter are inhibited; consequently there may be a tendency towards the delay of oestrus until the yield has fallen considerably below the potential level—in other words a greater delay in persistent, than in non-persistent, cows.

It will be seen, also, that with 1st calvers there is evidence of two "waves" in the means, and all or any of these factors might lead to this result; no adequate interpretation of the significance of the points (4 months and 8 months) at which the breaks occur in these curves can be offered.

Under these circumstances it would be very difficult to decide on a reliable curve to correlate S.F. with the length of the S.P.; a negative correction can be obtained from the mean S.F. of cows with S.P. of 300 days, but it is obvious that this would be numerically too large for the average cow with S.P. falling in the early intervals—for the mean S.F. concerned is 1·35, whilst all the indications are that the average cow in the early intervals has an upper limit of about 1·20; thus there are possible cases for which corrections obtained from these figures would be quite wrong, but, for the above reasons, these cases rarely arise in practice —the large negative correction for 300 days would not apply to the average cow, but would be approximately correct for those cows having such a long S.P.; consequently, for purposes of working out corrections to standardise S.F.'s for this factor, these means will suffice, since it seems that, if three separate curves were fitted to the "waves," they would all give roughly equal values for the standard S.P. (which is taken at 85 days—the normal length). It was therefore decided that correction tables might be obtained from the means in Fig. 1, which, although inaccurate as a reflection of the physiological relation concerned, would be approximately correct all through, for the great majority of cases which occur; no means show large deviations from the regression lines, and consequently the corrections given in Table II have been obtained from the latter—since the corrections were chiefly required for the present study, and are of limited value elsewhere, space is economised by giving them at 20-day intervals from 0–100 days, and

thereafter at 50-day intervals; the equations of the regression lines were

1st calvers ... ... S.F. = 0·932 + 0·00218 S.P.
Others ... ... S.F. = 0·817 + 0·00197 S.P.

Table II. *Corrections to S.F. for length of S.P.*

Standard S.P. = 85 days.

Length of S.P. in days	Percentage corrections		Length of S.P. in days	Percentage corrections	
	1st calvers	Others		1st calvers	Others
0	+19·8	+20·4	200	−18·3	−18·8
20	+14·5	+14·9	250	−24·4	−24·9
40	+ 9·6	+ 9·8	300	−29·6	−30·1
60	+ 5·1	+ 5·2	350	−34·1	−34·7
80	+ 1·0	+ 0·9	400	−38·1	−38·7
100	− 2·9	− 3·0	450	−41·6	−42·2
150	−11·3	−11·6	500	−44·8	−45·4

The two sets are practically identical—all over the range of S.P. from 0–500 days the difference between the figures never exceeds 0·6—and so the use of two tables was thought to be unnecessary; they were averaged and by their application all S.F.'s were standardised in respect to the length of the S.P.

Since the S.F. is calculated by dividing the total lactation yield by the maximum weekly yield and a constant (averaging very nearly 27), it was possible to calculate the mean maximum for each S.P. interval; this has been done with the results shown in Table III[1]. With both groups the first two means are low; the 40–59 days interval also gives means slightly below the average, but thereafter they fluctuate about a constant level. This is the form of relationship that might be expected, and the low mean maxima associated with early service may be due to two factors:

(1) A certain proportion of these cows would possibly have gone on increasing in yield and attained higher maxima had pregnancy not supervened so early in the lactation.

(2) Service may not be so easily accomplished very soon after calving if the production is high at that time; thus it appears probable that the recurrence of oestrus is influenced both by the absolute yield level, and also by level relative to the potential production of the cow concerned (cf. p. 151).

[1] In this table it has been taken that $27 \times \text{Mean maximum} = \frac{\text{Mean lactation yield}}{\text{Mean S.F.}}$; this of course is not strictly true; the result should be multiplied by $(1 - rv_1v_2 + v_2^2)$ where $v = \sigma/M$ and the suffixes 1 and 2 refer to lactation yield and S.F.(17); this bracket, however, is probably quite constant over all S.P. groups and so would not vitiate the comparison of groups; the inaccuracy in any case is small—substituting the values for raw lactation yield and S.F., it reduces to 1·013245.

Table III. *Calculated mean maximum by S.P. groups.*

	1st calvers		Others	
S.P. group	No. of observations	Maximum (lb.)	No. of observations	Maximum (lb.)
0–	10	181·9	23	233·4
20–	124	196·8	490	256·7
40–	169	199·8	703	268·2
60–	175	202·7	627	273·5
80–	115	206·6	409	276·0
100–	82	203·2	268	283·9
120 and over	206	205·9	519	275·5

Age.

The age variations in maximum and S.F. have been determined in two separate ways; in the first, simple correlation tables were drawn up between age, on the one hand, and maximum and S.F. on the other, and the means of the last two found for each age group—this has been called the "lumped lactations" method; in the second, the mean change from the 1st to the 2nd lactation was found from the records of all cows with these lactations known, similarly for the change from the 2nd to the 3rd lactation and so on, a curve being built up in steps—this has been called (by Roberts(9)) the "paired lactations" method. The two calculations do not necessarily give exactly similar results—in fact they bear on different problems, for the first gives the means for the actual populations at the various ages, whilst the second measures the rate of change in the physiological functions involved; thus the differences between the two sets of results must be caused by changes in the population—that is by selection, either deliberate by the farmer, or due to factors more or less outside his control.

Fig. 2 shows the relations concerned. The top two curves represent the variation in maximum weekly yield with age, the broken curve being fitted to the points obtained by pairing (solid circles) and the dotted curve to the means given by lumping (crosses); in both cases the yields are relative, that for 1st calvers being taken as 100. The next two curves are for total lactation yield, the dotted line, again, being the one obtained by lumping; the fit of these two curves has already been discussed(12), so that the points given by the data have been omitted. The other two curves represent the variation in S.F., the dotted one, as before, being that obtained by lumping; these differ from the others in not being fitted curves—they were obtained by connecting up the points given by the data; from the lumped correlation table 1st calvers gave a mean S.F. of

1·116, and this has been taken as the starting point for the other curve, with the object of making the two readily comparable.

The area between each pair of curves has arisen as a result of selection, and in the figure has been emphasised by vertical shading; it will be realised that selection at any age must be measured by the rate of divergence of the curves at that time, the actual distance between them being the net result of all previous selection.

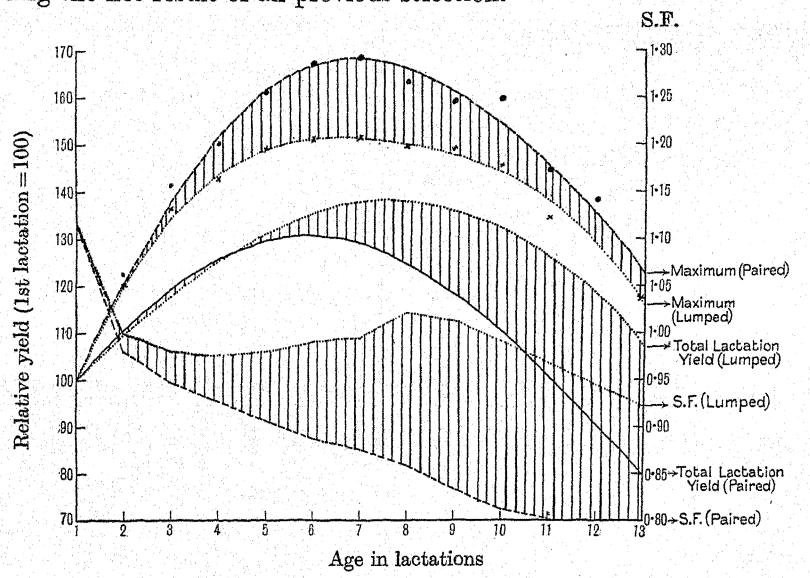


Fig. 2. Variations of total lactation yield, maximum weekly yield, and S.F., with age.

With maximum yield it is seen that the effect of selection is negative throughout; the curves diverge farther and farther from the 2nd to the 7th lactation and thereafter converge, so that in the earlier period, the cows that are discarded must tend to be those giving a high initial output, whilst the reverse must occur at the later ages; possibly the early negative relation may be due to a greater incidence of diseased conditions among cows with the capacity for a high rate of yield (this is commonly believed by farmers to be the case, but no definite evidence of it has been produced as yet) which more than nullifies any conscious selection that is practised; by the 7th lactation (9 years of age) the majority of the susceptible animals may have been weeded out, and so the conscious selection becomes apparent, though, as the figure shows, it never succeeds in making the net effect positive.

In the case of persistency selection is very definitely positive all

through; it is shown by the divergence of the curves from the outset, a divergence which continues up to the 9th lactation, after which they are approximately parallel. It is difficult to believe that this is entirely caused by deliberate disposal of non-persistent cows—rather it is probable that they are the unhealthy ones most liable to wastage through disease; it will be seen (below) that there is a slight negative correlation between maximum and persistency, so that the positive selection for persistency may play a small part in determining the negative selection for maximum.

A high maximum is more spectacular than high persistence, so that selection would be expected to be more apparent in the former; but it may be that in that case discrimination is obscured (or even reversed) by "natural" selection, whilst with persistency this factor accentuates it; according to this view the combination of high maximum with low persistency must be regarded as an indication of poor constitution.

Selection, therefore, appears as a compound factor, and its total effect is seen as the apparent selection for lactation yield—with lactations 2 and 3 the farmer fights a losing battle, but after that he wins all along the line.

With lumped lactations (dotted lines) the maximum rises by a noticeably greater proportion (51 per cent. as against 38 per cent.) with age than the lactation yield; this is reflected in the drop in persistency for lactations 2 to 4, but with the subsequent rise in S.F. the other two curves approach each other again; with lactation yield the highest point is reached at 7·65 lactations as compared to 6·58 with maximum yield.

Turning from the functions as given by the actual populations surviving to those showing the physiological relations (the paired curves) it is seen that the magnitude of the age variation in maximum yield is more than twice that in lactation yield—the rise is 68 per cent. in the former case and 31 per cent. in the latter—and the highest point of the curve for maximum is not reached until 6·88 lactations, as opposed to 5·90 for lactation yield. Here we see that persistency decreases all through the cow's life—rapidly (12 per cent.) from the 1st to the 2nd lactation, and after that remarkably constantly, at about 2 per cent. per lactation.

The main purpose of this part of the work was to analyse the function relating lactation yield to age, and the conclusions may be stated as follows: As age increases the maximum yield rises rapidly, but the lactation yield only rises about one-half as fast, being held back by persistency, which declines; this latter leads to a falling away of lactation yield before the maximum has attained its greatest value, and thereafter

the lactation yield decreases more rapidly than the maximum because persistency continues to decline. This is believed to be a true description of the changes experienced by individual cows, but the same conclusions would not be reached by studying lumped lactations, since populations showing biassed differences with age are involved.

The bottom curve in Fig. 2 provides a means of standardising S.F.'s in respect to age, and the correction factors derived from it are shown in Table IV. The standard was chosen so that after correction the distribution of S.F.'s might still centre around unity, as it was deemed undesirable to lose the clear-cut conception that figures above that value denoted persistency above the normal; unfortunately the standard did not correspond exactly to any particular lactation (though closely approximating to the 2nd), and it was regrettable that it differed from the standard to which lactation yields had been corrected; these disadvantages, however, were not thought so serious as the loss of the simple meaning of the S.F., which is inherent in its definition.

Table IV. *Corrections to standardise S.F. for age.*

Lactation	Percentage correction	Lactation	Percentage correction
1st	−11·4	7th	+13·0
2nd	+ 1·0	8th	+15·1
3rd	+ 4·4	9th	+18·6
4th	+ 6·7	10th	+21·8
5th	+ 9·5	11th	+23·9
6th	+11·5	12th	+26·1

The age variation in S.F. is in the reverse direction to that in maximum, but the forms of the functions are by no means reciprocal, so that the former cannot have arisen as a result of the latter acting in conjunction with a negative correlation between maximum and S.F.; correlation tables between the last two gave the following:

1st calvers ... ... $r = +0{\cdot}007 \pm 0{\cdot}023$;
2nd calvers ... ... $r = -0{\cdot}118 \pm 0{\cdot}024$;
Others ... ... $r = -0{\cdot}161 \pm 0{\cdot}014$.

There is, therefore, a slight negative correlation between maximum and persistency, but the values of r are so low that it could have no appreciable influence on the functions relating these to other variables; in the case of the 1st calvers r vanishes, and this may be due to an artificial reduction of the maximum caused by unfamiliarity with the act of milking, or by some other factor such as lack of development of milk cisterns.

The results described in this section are at variance with the conclusions reached by Gaines (5); with Guernsey cattle he obtained a correlation coefficient of + 0·480 between persistency and the initial rate of secretion (that is a negative association, since his k is an inverse measure of persistency as here defined), and when the latter was held constant the partial correlation between age and persistency was zero; Holstein-Friesian records confirmed this. It might be urged, however, that the total regressions were definitely non-linear and consequently the legitimacy of the partial correlation method was open to criticism; nor were the functions relating persistency and initial rate to age very closely similar, as would be anticipated if the one were entirely due to the other —in the case of Guernseys the fall in the value of k with the later ages was only evidenced by ten observations, whilst with Holstein-Friesians the highest point for k anticipated that for initial rate by 3 years. Of greater weight is the point that Gaines used the lumped lactations method —a method of questionable value in determining physiological relationships, because of the very strong (probably unconscious, and therefore universal) selection for high persistency.

Brody (1) came to similar conclusions to those of Gaines, but Turner (16) found that persistency declined with age, rapidly at first and then slowly, and stated that the curve representing this was a mirror image of that relating weight and fat production to age; he found no evidence of a rise in persistency with old cows.

Dry period.

The effect of the length of the D.P. on the total yield in the next lactation has already been described (12); in that study it was shown that a greater influence was exerted in the case of 2nd calvers than with older cows, and consequently the effect of the D.P. on the S.F. has been investigated separately in these two classes. In any enquiry into this factor the method of lumped lactations is utterly useless, since good cows exhibit a strong tendency to short D.P.'s and bad cows to long ones— there was, in these data, a correlation coefficient of $-0{\cdot}327 \pm 0{\cdot}012$ between D.P. and corrected lactation yield; it is evident from this that the only means of approach is by comparisons between the records of individual cows.

Taking the case of cows other than 2nd calvers first, the method of working was as follows: the D.P. distribution was split up into 7 intervals, 0–19 days, 20–39 days, etc., all over 120 days being put together;

comparisons were then made between the S.F.'s of individual cows after D.P.'s falling in different intervals—thus 6 intervals were compared to 0–19 days, 5 more to 20–39 days, and so on, making 21 comparisons in all. The results are shown in the first part (columns 1–6) of Table V; with each series of comparisons the mean S.F. for the lowest interval has been placed at 100, and the means given by the comparisons of the others with that interval are shown accordingly. It will be noticed that of the 21 figures obtained 18 are greater than 100—that is to say, in only 3 cases was the mean S.F. following the longer D.P. less than that after the shorter one; assuming that D.P. has no effect on S.F. the signs of these differences would be distributed at random, and in a long series of trials the number of cases where all were of the same sign, all but one of the same sign, etc., would be proportional to the terms in the expansion of $(\frac{1}{2} + \frac{1}{2})^{21}$; the sum of the first 4 terms of this binomial is 0·000745, so that the odds against so few as three of these differences favouring the shorter D.P. are 0·999255 to 0·000745, or 1341 to 1; it is permissible therefore to draw the conclusion that a long D.P. tends to raise the S.F. in the next lactation.

Table V. *Effect of length of D.P. on the S.F. in the next lactation.*

All cows other than 2nd calvers.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
	D.P. compared with that on the left (days)						Percentage difference from figure next above. D.P. intervals (days)						Mean of columns	Rela-
D.P. (days)	0–	20–	40–	60–	80–	100–119	0–	20–	40–	60–	80–	100–119	7–12	tive S.F.
0– 19	100·0	—	—	—	—	—	—	—	—	—	—	—	—	100·0
20– 39	99·8	100·0	—	—	—	—	−0·2	—	—	—	—	—	−0·2	99·8
40– 59	99·9	103·2	100·0	—	—	—	+0·1	+3·2	—	—	—	—	+1·65	101·4
60– 79	107·6	105·5	101·6	100·0	—	—	+7·7	+2·2	+1·6	—	—	—	+3·8	105·3
80– 99	111·8	105·7	106·1	102·2	100·0	—	+3·9	+0·2	+4·4	+2·2	—	—	+2·7	108·1
100–119	109·0	104·0	102·7	103·4	100·9	100·0	−2·5	−1·6	−3·2	+1·2	+0·9	—	−1·0	107·0
120–	105·3	108·4	106·8	113·6	106·7	94·8	−3·4	+4·2	+4·0	+9·9	+5·7	−5·2	+2·5	109·7

(Mean = 150 days)

The next step was to condense the figures to such a form that the function sought might appear; in the second part of the table (columns 7–12) the figures in the first part are expressed as percentage differences from those next above them—thus column 1 shows that if S.F.'s following D.P.'s of 0–19 days be taken as 100, the comparisons with D.P.'s of 20–39 days and 40–59 days gave relative mean S.F.'s of 99·8 and 99·9 respectively; proceeding from the first to the second interval there is a drop of 0·2 per cent., and from the second to the third a rise of 0·1 per cent., and consequently these figures appear in column 7. In this way we arrive at a series of estimates of the changes in S.F. as we pass down the table, and column 13 gives the arithmetic mean of these; it might

have been better to weight these figures before taking the mean, possibly choosing weights inversely proportional to the standard errors of the differences—that is, weights equal to $\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$, where n_1 and n_2 are the number of observations in the two comparisons concerned; such a procedure, however, would presuppose a constant standard deviation of difference over all comparisons, which is probably not true; if the figures in the second part of the table are received as estimates, the taking of the much simpler arithmetic mean can be defended.

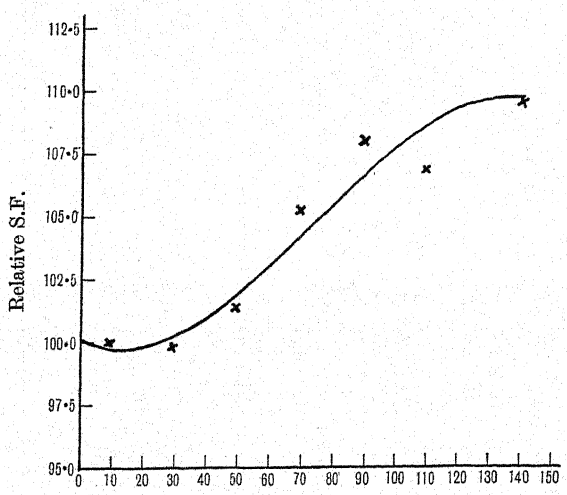


Fig. 3. Effect of length of D.P. on S.F. Cows other than 2nd calvers.

Starting from 100 and applying the percentages of column 13 successively, we arrive at column 14, which is regarded as giving at least a first approximation to the function relating the S.F. to the length of the preceding D.P. The following curve was fitted to these points by the method of least squares:

$$y = 100{\cdot}13 - 0{\cdot}0619.x + 0{\cdot}00255.x^2 - 0{\cdot}0000115.x^3,$$

where y = comparative S.F., and x = D.P. in days. This curve is shown in Fig. 3; its fit is only fair since the points are somewhat scattered, but it seems to represent the relationship sufficiently closely, within the limits of the experience—but it is quite clear that it would not allow of extrapolation.

The curve portrays a peculiar relationship; with D.P.'s from 0 to 40 days there is no appreciable effect, but from that point up to 120 days the S.F. rises in an approximately linear manner, after which it appears to remain constant. This is by no means similar to the curve depicting the association between lactation yield and D.P. (12), which rises rapidly at first and then flattens out, approaching a horizontal asymptote.

With 2nd calvers a completely different method was employed; S.F.'s having been corrected for S.P. and age the differences between the figures given by the 1st and 2nd lactations of particular cows were regarded as due to the intervening D.P., and consequently a correlation table was drawn up between these two; as an example one cow gave corrected S.F.'s in the two milking periods of 1·10 and 1·19, the D.P. being 80 days —here the S.F. difference was + 8 per cent., and this was correlated with a D.P. of 80 days. In all there were 429 observations, and they gave the results shown in Table VI.

Table VI. *Variation of S.F. with D.P. 2nd Calvers.*

D.P. (days)	No. of observations	Mean percentage difference from S.F. in the cow's 1st lactation	20-day groups —relative S.F.'s with 0–19 days D.P. as 100
0–9	51	− 0·55	100·0
10–19	54	− 1·89	
20–29	80	0·00	99·5
30–39	51	− 4·47	
40–49	44	− 2·43	100·1
50–59	28	+ 0·75	
60–69	20	+ 1·75	106·2
70–79	29	+ 7·00	
80–89	19	+ 6·74	110·2
90–99	15	+11·67	
100–109	15	+ 8·00	106·8
110–119	5	− 2·00	
120 and over	18	+12·42	113·8

This variation is very closely similar to that shown in Table V for older cows; again there is little or no effect evident in the lower D.P. groups, and a definite rise from 40 to 120 days; with somewhat limited data in each case and consequent irregularity of the means, slight differences between the two sets of figures might well be due to chance, but we note that with 2nd calvers the flat part of the curve would extend a little farther, and the subsequent rise would be steeper, the curve apparently attaining a value of approximately 111 at 120 days—there is, in fact, little evidence of any approach to a maximum, and it cannot be concluded that it does not keep on rising indefinitely with very long D.P.'s, since we have no experience in that region of the table.

For later work it was required to eliminate the effect of D.P. on S.F., and for this purpose the figures given in Table VII were used; taking a standard D.P. of 40 days no corrections are necessary for short D.P.'s, but with longer ones a percentage must be subtracted; it is seen that the effect is not a very large one, and that the corrections are slightly more steeply graded in the case of 2nd calvers (Table VII).

Table VII. *Corrections to standardise S.F. for D.P.*

Standard D.P. = 40 days.

D.P. (days)	Correction: 2nd calvers (%)	Correction: Others (%)	D.P. (days)	Correction: 2nd calvers (%)	Correction: Others (%)
40–49	−0·5	−1·5	90– 99	− 7·0	−6·9
50–59	−2·0	−2·8	100–109	− 8·2	−7·7
60–69	−3·0	−3·6	110–119	− 9·1	−8·5
70–79	−4·4	−4·8	120 and over	−10·0	−9·0
80–89	−5·9	−5·9			

As before, the next step was to analyse the effect of this factor on lactation yield into its effects on maximum and persistency; since lactation yield is proportional to the product of the maximum and the S.F. we have the following relative figures.

	D.P. = 0	D.P. = 40	D.P. = 120
(*a*) Cows other than 2nd calvers:			
Lactation yield	100	118	131
S.F.	100	101	110
Maximum	100	117	119
(*b*) 2nd calvers:			
Lactation yield	100	132	153
S.F.	100	100	111
Maximum	100	132	138

Whence we see that the earlier and later portions of the D.P. are responsible for the following increases in S.F. and maximum (percentages of their values for D.P. = 0):

	0–40 days		40–120 days	
	Other than 2nd calvers	2nd calvers	Other than 2nd calvers	2nd calvers
S.F.	1	0	9	11
Maximum	17	32	2	6

This pictures the effect of the D.P. on the next lactation curve thus: As a result of the first 40 days the whole curve is put up in the same proportion, the amount by which it is raised with 2nd calvers being about double that for older cows; at about this point some limiting factor comes into play and further rest has but little influence on the maximum (particularly with older cows) but finds expression in tilting up the re-

mainder of the curve, and despite the longer continued rise in maximum this tilting is slightly greater with 2nd calvers.

The final physiological interpretation of this must be left to the experimentalist; two possibilities are apparent:

(1) The maximum may have an upper limit determined by the area of the gland available for secretion, and the full development of this may require an interval of inactivity between milking periods: a rest of 6 weeks may be sufficient for the practical completion of this, and thereafter the only benefit accruing from rest is due to its permitting a storage of food materials, which tends to keep the yield up later in the lactation; this latter is in accord with the conclusions of Eckles and Palmer (3).

(2) The storage of food materials may be the main factor all through; if very little takes place the full expression of the gland area is prevented, and the maximum is held down in the same proportion as the yield during the rest of the lactation; on the average, 6 weeks' storage is sufficient to support the maximum activity for a short time, and, this having reached its limit, further storage is only evidenced by the staving off of the subsequent fall.

The former would appear more probable from our knowledge of glandular growth, and is quite in accord with the greater effect observed in the case of the 2nd calvers—in the other view, this must be due to the increased demand on nutriment caused by growth. Neither of these takes into account any influence which a rest may have on the rate of senility of the mammary cells in the next lactation—this is a question of which we know very little. Further facts bearing on this problem are given in Part II (pp. 168–73), which, though far from conclusive, lend some support to the view that the D.P. effect is a double one in time—first glandular growth, in general overshadowing nutrition, and then nutrition alone.

Part II. The problem of standardisation at different levels of persistency.

Perfection may be unattainable but this does not detract from the importance of knowing where we fall short of it—in fact here lies the difference between that little knowledge which is reputed to be dangerous and a fuller conception which may be useful. In the present case it is freely admitted that an exact correction system for milk records, based on the persistency level of the cow whose record is to be standardised, would be unwieldy and intricate, and would involve so much arithmetic of a rather specialised kind that it would never conceivably find any

place in practice; it would appear that standardisation must always be based on the average cow, but the proper appreciation of the individual case necessitates a realisation of the errors involved; in this Part an attempt is made to show the directions in which errors lie in the correction of the records of persistent and non-persistent cows. In the case of month of calving and S.P. the 3918 lactations have been divided into two groups according to the S.F. corrected for S.P. only—that is, according to the persistency level in the lactation concerned; with age and D.P. the effects cannot be determined (see Part I) by the lumped lactations method, and so other devices have to be introduced, which lead to a severe restriction of the field of enquiry.

Month of calving.

The distribution of "S.P. standardised" S.F.'s gave a median of 1·011, and consequently (S.F.'s being always calculated to two decimal places) all those lactations giving figures of 1·02 and over were taken as high S.F.'s, and the remainder as low S.F.'s; these two groups gave the following:

	High S.F.'s	Low S.F.'s
N	1943	1975
Mean S.F.	1·155±0·002	0·874±0·002
Standard deviation of S.F.	0·112±0·001	0·103±0·001
Mean lactation yield (standardised for S.P., age and D.P.)	8799±32 lb.	7015±26 lb.

The mean lactation yields for the different months of calving, with the two groups, are shown in Fig. 4 (the months January to April being repeated at the right so that a view of the general trend at any point may be obtained), and the correction factors found from these means are given in Table VIII.

Table VIII. *Corrections to eliminate the month of calving effect.*

Month of calving	Percentage corrections: High S.F.'s	Low S.F.'s	Month of calving	Percentage corrections: High S.F.'s	Low S.F.'s
January	+0·7	− 3·8	July	+1·0	+7·6
February	−0·1	− 5·4	August	−0·3	+8·5
March	−0·2	− 0·9	September	+0·9	−1·4
April	−2·8	− 0·8	October	−4·7	−5·5
May	+3·3	+ 5·5	November	−1·0	−3·4
June	+5·4	+10·0	December	−1·4	−6·5

It is at once apparent that these two groups differ in their reaction to this factor. In the high persistency group the whole variation is toned down, so that there are only three months which show any considerable deviation—October is a favourable month for calving, and May and June are unfavourable; in addition to these April calvers give

a high mean, which appears as an irregularity in the curve, and this may be a chance fluctuation, though of this we cannot be certain. With the low persistency group the curve is very regular, only minor fluctuations disturbing the general trend; it shows a rounded form in the autumn months, which are the best, with December as the optimum. It is interesting to notice the fact that the most favourable month comes earlier with high S.F.'s (October), which might be expected since such cows are

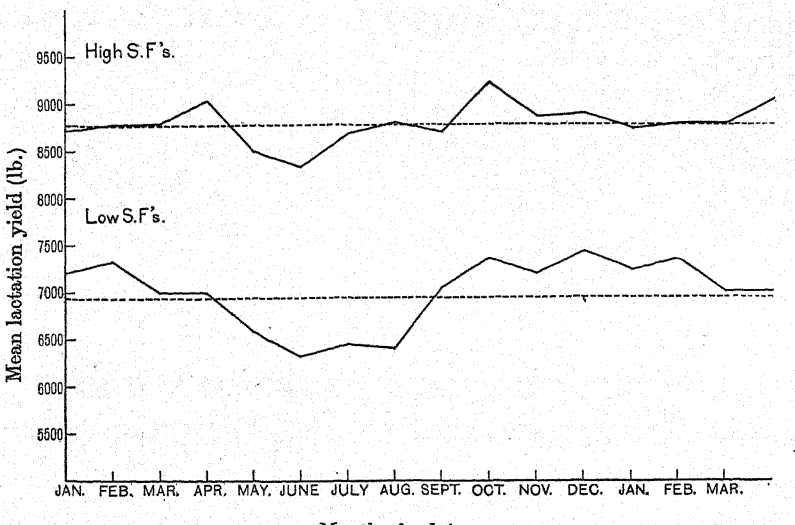


Fig. 4. Effect of month of calving on lactation yield—high and low S.F.'s.

capable of deriving appreciable benefit from the spring flush at a later stage of the lactation than are the other group; this fact is also seen to mitigate against the unfavourable factors associated with late summer calving (discussed elsewhere (12)), with the result that July and August are raised almost to the mean.

Table VIII brings out the important point that the influence is slight with persistent cows, compared to the others; a rough measure of the proportionate variation caused by this factor might be obtained by adding up the corrections regardless of sign, and if this is done it is seen that the variation with high S.F.'s is but little more than one-third of that with low S.F.'s; this, again, might be anticipated for with non-persistent cows the yield is more concentrated and so liable to more violent fluctuations; with persistent cows the yield is better distributed over a number of months, so that there is a greater chance of compensation.

Service period.

For determining the effect of the length of the S.P. on the lactation yield at different levels of persistency, correlation tables were drawn up between these variables from the two groups mentioned in the last section. These gave the following constants:

	High S.F.'s	Low S.F.'s
r	$+0{\cdot}414 \pm 0{\cdot}013$	$+0{\cdot}380 \pm 0{\cdot}013$
η	0·436	0·398
$N(\eta^2 - r^2)$	36·334	27·650
$a-2$	15	15
P	<0·01	0·026

With persistent cows, therefore, there is a higher correlation coefficient between S.P. and lactation yield than with non-persistent cows, but the difference is not significant; with the former the regression is definitely non-linear, but with the latter we cannot be quite certain of this. The actual means obtained for the different S.P. intervals are shown in Fig. 5, the scale being moved up slightly for high S.F.'s to avoid intermingling; an attempt was made to fit curves to these points but the scatter is so great with the long S.P. intervals that it was felt that no fitted curves could be trusted with any confidence; consequently the smoothing of the points was left to the regression lines, which, as the figure shows, cut through them quite well, despite the disagreement between r and η. From the regressions standardising corrections were obtained, and these are shown, in an abridged form, in Table IX; they are numerically less throughout for low S.F.'s, but the differences are so small as to be negligible.

Table IX. *Corrections to standardise lactation yield for length of S.P.*

S.P. (days)	High S.F.'s (%)	Low S.F.'s (%)	S.P. (days)	High S.F.'s (%)	Low S.F.'s (%)
0	+22·3	+21·1	100	− 3·1	− 3·0
20	+16·2	+15·4	200	−19·8	−19·1
40	+10·7	+10·1	300	−31·5	−30·6
60	+ 5·7	+ 5·4	400	−40·3	−39·2
80	+ 1·1	+ 1·0	500	−47·1	−45·9

The absence of any marked difference in this respect between the two groups was very surprising; it had been confidently anticipated that non-persistent cows, having a higher proportion of their yield in the early part of their lactation, would give higher positive corrections for short S.P.'s, and lower negative ones for long S.P.'s; this, in fact, has been held to explain the difference between 1st calvers and older cows in their S.P. variations (12). It is possible that the failure of the result

to emerge may have been due to the selection which is operating over the S.P. range (discussed in Part I); cows with long delayed service all tend to be persistent yielders, though the severe correction to S.F. for S.P. arising out of this has made a proportion appear here among the low S.F.'s (two types of persistency are common—the level curve from the maximum awards, and a considerable fall for a short time, followed

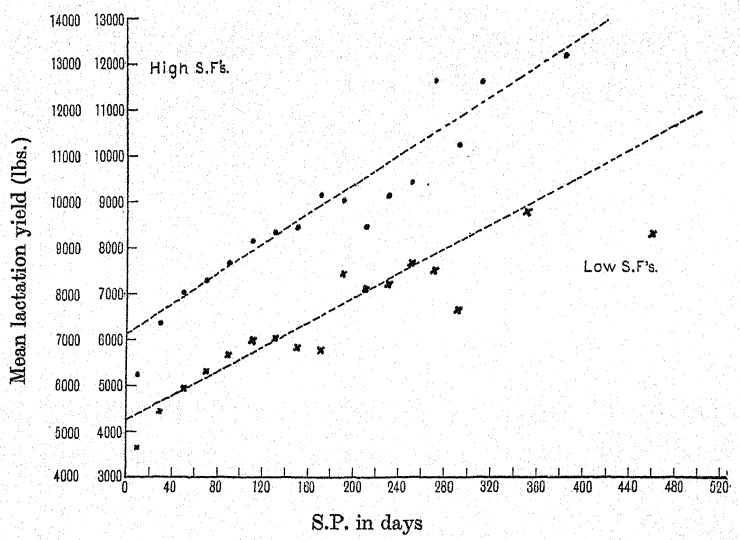


Fig. 5. Effect of length of S.P. on lactation yield.

by a very level output: these, presumably, were of the second type). This selection was apparent (Fig. 1) from the S.P. interval 140–159 days upwards, so that the intervals lower than this were not appreciably disturbed by it; taking the actual means for the latter, and calculating the figure for the standard S.P. of 85 days by linear interpolation, the following corrections were obtained:

S.P. interval (days)	High S.F.'s (%)	Low S.F.'s (%)
0–19	+44·5	+42·1
20–39	+19·1	+21·4
40–59	+ 8·1	+10·8
60–79	+ 4·0	+ 4·8
80–99	− 1·1	− 1·3
100–119	− 7·2	− 5·5
120–139	− 8·9	− 5·9

Here we see exactly the difference that might have been expected, there being only one exception, namely, in the lowest interval, for which there were only eight observations with high S.F.'s; nevertheless the

results described in this section must be regarded as unsatisfactory, and it cannot definitely be claimed that any difference between persistent and non-persistent cows, in their reaction to the S.P. factor, has been demonstrated.

Age.

In the case of this factor the investigation was confined to 84 cows, owing to the difficulties in dividing the cows—as distinct from the lactations—into a persistent and a non-persistent group (exactly similar difficulties in obtaining high and low lactation yield groups from these data have been discussed elsewhere(12)); the individuals included were all those of which lactations 1–4 were included in the data, and they were divided into two groups according to the mean corrected S.F. in the four lactations. These groups gave the following actual and relative mean yields (corrected for month of calving and S.P.):

	Mean yield (lb.)		Relative yield	
Lactation	High S.F.'s	Low S.F.'s	High S.F.'s	Low S.F.'s
1st	6445	5017	100·0	100·0
2nd	7507	5945	116·5	118·5
3rd	7953	6746	123·4	134·5
4th	8039	7012	124·7	139·8

The difference between the two groups in their yield rise with age is closely similar to that found when these same cows were divided in like manner according to the mean lactation yield(12), high S.F.'s corresponding to high yielders and low S.F.'s to low yielders; this, of course, follows from the fact that high S.F. is usually associated with high lactation yield. In the present case the two groups show rather less divergence in their yield levels—since the cows were assigned to them by only one of the factors making for high yield, with no reference to their maxima—but nevertheless the greater proportional rise of low S.F.'s over high S.F.'s is accentuated as compared to that of low yielders over high yielders. The greatest distinction appears to lie, therefore, between cows of different persistency level, rather than cows of different lactation yield level; the conclusion follows that heifers showing low persistence may be expected to increase in their yields by a greater proportion in subsequent lactations, than heifers with high persistence, but that the same does not apply to maximum yield; if, then, a heifer has a low production to her credit in her first lactation, it is more likely to prove worthy of a further trial if this is due to low persistency, than if it is due to low maximum; the proviso should, perhaps, be added, that the evidence for this rests on "commercial" data, and that it may not apply

to Advance Registry animals where the nutrition is on a much higher plane.

Dry period.

It was only with 2nd calvers that a satisfactory method of forming a high and a low S.F. group, for the study of the effect of the length of the D.P. on the lactation yield, could be found, so that the present section is confined to such animals. The data contained records of the 1st and 2nd lactations of 429 cows; the corrected S.F.'s in these lactations were averaged for each individual and of these averages 218 were 1·00 or over, and 211 were less than 1·00, so that these were taken as the high and low S.F. groups.

The method of working was to take all lactation yields standardised for month of calving, S.P., and age, to express each 2nd lactation yield as ± a percentage of that cow's 1st lactation yield, and to correlate these percentages with the length of the D.P. between the two milking periods; the two correlation tables gave the following statistical constants:

	High S.F.'s	Low S.F.'s
N	218	211
r	$+0{\cdot}436 \pm 0{\cdot}037$	$+0{\cdot}486 \pm 0{\cdot}035$
η	0·596	0·596
$N(\eta^2 - r^2)$	35·996	25·113
$a-2$	11	16
P	<0·01	0·071

In both cases there is a significant positive correlation showing that the lactation yield rises as the D.P. increases; the values of $N(\eta^2 - r^2)$ indicate that the regression of yield on D.P. is linear in the case of low S.F.'s but certainly non-linear with high S.F.'s, which is borne out by the means for the various D.P. intervals shown in Fig. 6.

It is quite definite that the form of the relationship is not the same in the two groups; with persistent cows the means rise very steeply at first, but at about 40 days D.P. the rise practically ceases, the three remaining means lying nearly horizontally; the trend might be represented quite closely by two straight lines, but the hyperbola

$$y = \frac{116{\cdot}2x + 335{\cdot}3}{x + 7{\cdot}0}$$

has been fitted; this cuts through the points very well and seems to represent the function connecting the two variables, down to a D.P. of about 5 days—below that, however, its fall would be excessively steep and, in the figure, it is not continued. With non-persistent cows the figure shows that the regression line $y = 79{\cdot}55 + 0{\cdot}342.x$ gives a satisfactory fit to the data.

It follows from the above that D.P. corrections must always be in the nature of a compromise, unless the persistency level of the cow is taken into consideration; this is demonstrated in Table X, which gives the D.P. corrections derived from Fig. 6 for high and low S.F.'s, and also those obtained by putting all these cows together (12).

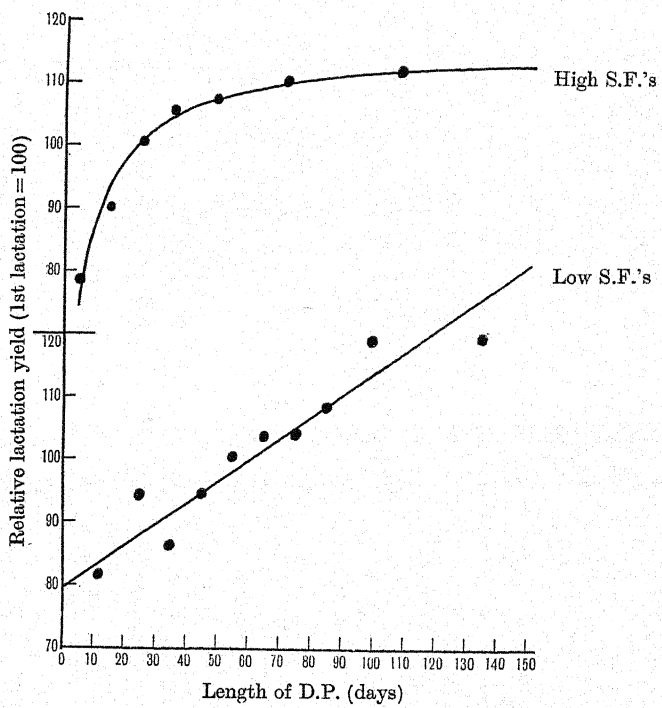


Fig. 6. Effect of the length of the dry period on the lactation yield—2nd calvers.

Table X. *Corrections for length of D.P.*

Standard D.P. = 40 days.

D.P. (days)	High S.F.'s (%)	Low S.F.'s (%)	All cows together (%)
0–9	+42·1	+14·9	+25·1
10–19	+12·8	+10·3	+15·2
20–29	+ 5·0	+ 6·0	+ 8·0
30–39	+ 1·2	+ 2·0	+ 2·8
40–49	− 0·8	− 1·7	− 1·3
50–59	− 2·2	− 5·1	− 4·4
60–69	− 3·2	− 8·3	− 6·4
70–79	− 3·9	−11·3	− 8·8
80–89	− 4·5	−14·1	−10·4
90–99	− 4·9	−16·7	−11·7
100–109	− 5·3	−19·2	−12·7
110–119	− 5·6	−21·5	−13·5

It is apparent that, by correction with figures obtained from lumping together cows of all persistency levels, those cows which give high S.F.'s will have an insufficient addition for D.P.'s of less than 10 days, will be credited with a little too much for D.P.'s from 10–40 days, and will be penalised too heavily for long ones; non-persistent cows, on the other hand, will gain more than their due for D.P.'s lower than the standard, and will have their yields insufficiently reduced for longer D.P.'s. Any simple correction system must disregard errors of this type, but their existence should be realised.

Before leaving this question it was thought desirable to probe a little deeper in an effort to gain further insight into the above difference between persistent and non-persistent 2nd calvers; accordingly D.P. was correlated with S.F. in the high and low S.F. groups—that is, the correlation table which gave rise to Table VII was divided into two on the same basis as used in this section; the two sub-tables gave the following:

	High S.F.'s	Low S.F.'s
r	$+0{\cdot}068 \pm 0{\cdot}045$	$+0{\cdot}390 \pm 0{\cdot}039$
η	0·202	0·451
$N(\eta^2 - r^2)$	7·887	10·824
$a-2$	9	14
P	0·547	0·700

With high S.F.'s, r and η are both quite insignificant, but with low S.F.'s they are approximately $+0{\cdot}4$; η^2 does not differ significantly from r^2 in either case, so that the regressions are linear—their equations are

$$\text{High S.F.'s: } y = 100{\cdot}48 + 0{\cdot}043x;$$
$$\text{Low S.F.'s: } y = 86{\cdot}52 + 0{\cdot}218x;$$

where y = relative S.F. in 2nd lactation (S.F. in same cow's 1st lactation = 100), x = D.P. before the 2nd lactation (in days).

In the case of high S.F.'s the regression coefficient, like r, was, of course, insignificant. Fig. 7 shows the two series of means together with the regression lines.

On p. 161 the D.P. effect was analysed, but it now becomes clear that, at least with 2nd calvers, that analysis only represents an average over all persistency levels; it appears that different limiting factors must be involved in the cases of persistent and non-persistent cows, and an attempt must be made to interpret the dissimilar results in the two groups.

Taking high S.F.'s first, Fig. 6 shows that their lactation yield is raised at an approximately constant rate of 0·75 per cent. per day of rest, from D.P. = 0 to D.P. = 40; the S.F. is unaffected (the actual (insignificant) rise shown in Fig. 7 is 0·043 per cent. per day) so that the

maximum must be raised in the same proportion. From D.P. = 40 to D.P. = 120 there is very little effect apparent; the lactation yield rises at 0·075 per cent. per day, and this is but little more than the rise in S.F., so that the maximum must have practically reached its limit at D.P. = 40.

With low S.F.'s there is no evidence of discontinuity, the lactation yield and the S.F. rising constantly throughout—the former at the rate of 0·342 per cent. per day and the latter at 0·218 per cent. per day; since

$$\frac{1{\cdot}00342}{1{\cdot}00218} = 1{\cdot}00123,$$

it follows that the maximum must rise constantly at the rate of 0·123 per cent. per day.

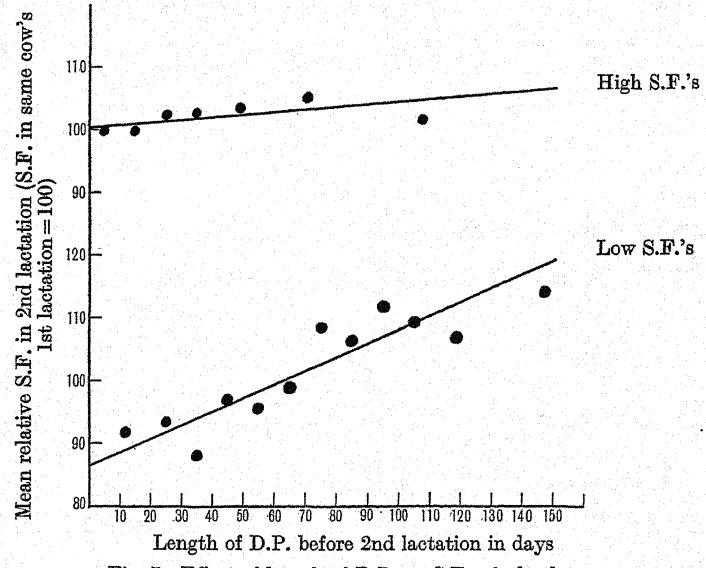


Fig. 7. Effect of length of D.P. on S.F.—2nd calvers.

A plausible explanation might be built up on the premise that the essential difference between persistent and non-persistent cows lies not in the rate of senility of the mammary cells but in the intermediary metabolism—that in the former the mammary gland is more efficient in competing with the other tissues of the body for the nutrients present in the blood; as to whether it is a peculiar property of the gland, or of the way in which the nutrients are prepared and distributed we have no possible indication.

In the case of the persistent milker there is a rapid rise in the maximum associated with the first 40 days of D.P., and it is concluded that

inactivity for such a period (and no longer) is necessary for the complete development of the gland; further rest has no value in increasing the next lactation yield since storage of nutrients has little or no influence, intermediary metabolism being favourable to milk production; with this cow, therefore, the limiting factor is glandular, and the rate of senility of the secreting cells as the lactation proceeds appears not to be affected by the length of the period of rest.

With the non-persistent milker, on the other hand, the fundamental limiting factor is nutritional, and this prevents the full expression of the glandular development in the maximum yield; under these circumstances the important point is the storage of food materials, and this affects the maximum and persistency roughly in the proportion 1 : 2 (123 : 218).

This view is in accord with the conclusion expressed on p. 161, that the D.P. effect is a double one—glandular development and storage, the former only requiring 6 weeks for its full conclusion, whilst the latter goes steadily on for a considerable period; the relative importance of these two factors seems to vary very widely from cow to cow, for with highly persistent milkers storage plays little, if any, part, whilst with cows at the other extreme it is paramount, completely obscuring the other.

Other facts might be adduced in support of the present conclusion; thus the slight negative correlation between maximum and persistency would be expected, since a high maximum means a rapid dissipation of food reserves, which alone enable the yield to be maintained when nutrition is unfavourable; the greater age increase of non-persistent cows, noted in the last section, would also be expected, since in the early lactations, with concomitant growth, storage is very limited. It will be seen that the foundation on which this interpretation rests is that the main physiological difference between persistent and non-persistent cows lies in intermediary metabolism; the former is the one in which the food goes to milk as opposed to beef; the writer has never seen any definite evidence that the dairy breeds are more persistent milkers than the beef breeds, but this is usually accepted as true—cf. Eckles (2), Meigs and Cary (10) and Turner (14)—while Brody (1) showed that scrub cows were definitely inferior in persistence. It must be admitted that the placing of the breeds as regards persistency (given in Part III) does not show dairy breeds to excel dual-purpose breeds; in the genesis of breeds, however, persistence may have been developed to varying extent, as it may very well be (in fact appears to be) genetically distinct from

maximum yield; thus breed peculiarities in this respect are conceivable, even among the true dairy breeds.

The alternative of introducing the senility concept does not seem to clarify the position very much, for senility, in our present state of knowledge, seems only to be a particular form of mal-nutrition, if the latter is given its widest meaning. There is something attractive in drawing the analogy between the senility of the mammary gland as lactation progresses and general senescence, and that the former should be more rapid with advancing age seems appropriate, but this does not carry us very far. Even more attractive is the possibility of linking up senility of the mammary gland with muscle fatigue, in both of which the two main factors appear to be the exhaustion of supplies and the accumulation of products.

PART III. THE RELATIONS BETWEEN MAXIMUM YIELD, PERSISTENCY AND LACTATION YIELD, AND THEIR RELATIVE CONSTANCY IN THE INDIVIDUAL.

Distributions.

Taking all lactations together the distributions of S.F. (raw and corrected) and maximum (raw) are given in Fig. 8; it is to be regretted that the method of working, by avoiding at each stage the calculation of corrections for maximum, did not provide the corrected distribution of that variable. The following constants were obtained:

	S.F.		
	Raw	Corrected	Raw maximum
Mean	1·012 ±0·002	1·013 ±0·002	256·8 ±0·72
Median	0·992 ±0·002	1·011 ±0·002	251·5 ±0·91
Mode	0·952	1·007	240·9
σ	0·213 ±0·002	0·180 ±0·001	67·10 ±0·51
v	21·05 ±0·17	17·81 ±0·14	26·13 ±0·21
Sk	+0·282 ±0·012	+0·033 ±0·013	+0·237 ±0·012

The coefficient of variation for raw maximum is significantly greater than that for raw S.F., and though both exhibit positive skewness in approximately equal degree, the distribution of maximum is appreciably more flat topped. By correcting S.F.'s the coefficient of variation is reduced and the skewness disappears; the normal curve corresponding to this distribution has the equation $y = 435{\cdot}3\,.\,e^{-x^2/0{\cdot}064466}$, and, testing the goodness of fit of this curve, $\chi^2 = 28{\cdot}481$, which, with 25 degrees of freedom, gives a value of P of 0·288; it appears, therefore, that inherent

capability as regards persistency is distributed according to the normal curve.

To obtain some light on the relative influences of maximum and persistency in determining lactation yield, the following correlations were determined:

Raw maximum × Raw lactation yield, $r = + 0{\cdot}700$;
Raw S.F. × Raw lactation yield, $r = + 0{\cdot}492$;
Corrected S.F. × Corrected lactation yield, $r = + 0{\cdot}566$.

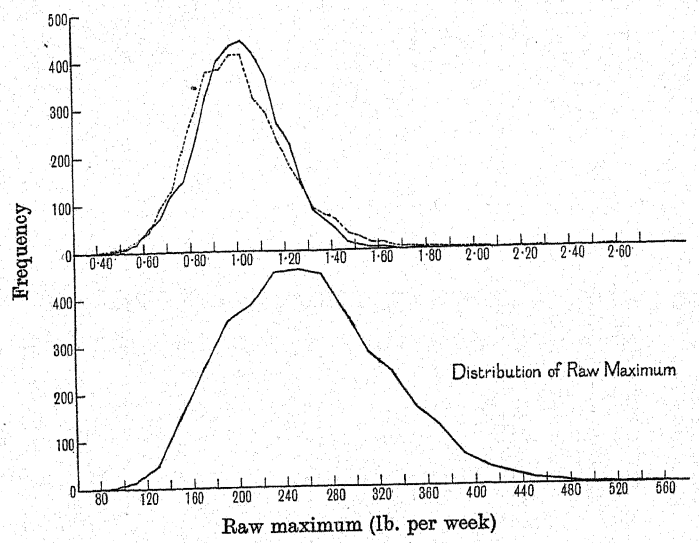


Fig. 8. Distributions of persistency and maximum (3918 lactations).
Distribution of S.F.'s: Raw - - - - - - - -. Corrected ————.

If y denote raw lactation yield, and y' denote lactation yield corrected by a regression equation, we know that

$$\sigma_{y'} = \sigma_y \sqrt{1 - r^2}.$$

Taking the raw figures and substituting the above values of r for maximum and persistency respectively, we have

$$\sigma_{y'} = \sigma_y \times 0{\cdot}714,$$

and

$$\sigma_{y'} = \sigma_y \times 0{\cdot}871.$$

Consequently, of the variation in lactation yield, 28·6 per cent. is accounted for by variation in maximum, as compared to 12·9 per cent. in the case of persistency. From lactation to lactation (there being varying

numbers of lactations of different individual cows) this indicates that maximum is just over twice as potent in determining lactation yield as is persistency, but that the same holds true from cow to cow does not necessarily follow; corresponding results for the latter case would be given by the corrected figures, but the correlation between corrected maximum and corrected lactation yield is lacking; much of the raw correlation between these two must be attributed to the similarity of their age variations (which is in the opposite direction with persistency), so that the preponderance of the maximum might be much less marked with corrected figures—that is in determining the variation from cow to cow; that this is actually the case is indicated by the results given later.

From the correlation between the corrected S.F. and the corrected lactation yield it was possible to work out the approximate mean and standard deviation of the distribution of corrected maximum[1]; with raw figures the calculation gave a mean of 257·3 lb. and a standard deviation of 68·0 lb., which are very close to the actual values given above. Substituting the corrected figures (and remembering that the standard for the S.F. is 11·5 per cent. above the value for the 6th lactation) the following were derived:

$$\begin{aligned} \text{Mean corrected maximum} &= 320{\cdot}3 \text{ lb.;} \\ \sigma &= 70{\cdot}2 \text{ ,, ;} \\ v &= 21{\cdot}93 \text{ ,, .} \end{aligned}$$

Thus the relative variability of inherent capacity for maximum yield is greater than that of persistency, in the ratio 21·93/17·81, or as 1·23 : 1.

Breed comparison.

The comparison of the yielding capabilities of the six chief breeds (as evidenced by the standardised yields) is made in Fig. 9, and is analysed into its two constituents, maximum and persistency; the means for the corrected maximum were obtained from the formula used above, namely,

$$\frac{1}{27{\cdot}1} \times \frac{M_1}{M_2}(1 - r.v_1v_2 + v_2^2),$$

where $v = \sigma/M$, and the suffixes 1 and 2 refer to lactation yield and S.F., respectively; the value of r, the correlation between corrected S.F. and corrected lactation yield, was taken as the same all through—+ 0·566, as found by taking all breeds together.

* See footnote to p. 152.

It is seen that Friesians owe their pre-eminence to high maximum, their persistency being only 2 per cent. above the average of all the breeds; Mongrels are slightly subnormal in maximum but show high persistency, whilst Friesian Crosses are just about on the average for both maximum and persistency—and consequently for lactation yield as well. Red Polls show very peculiar placing, being bottom in maximum but top in persistency, whilst Lincoln Reds show the opposite, approxi-

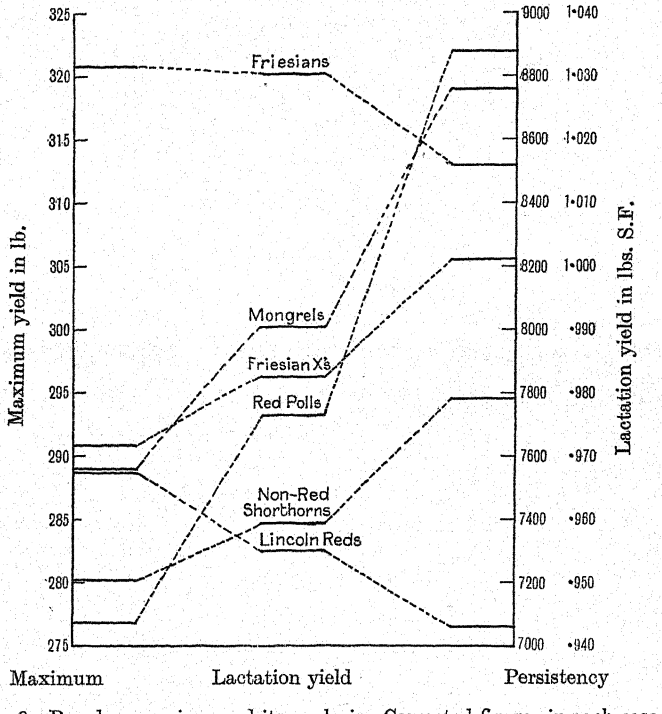


Fig. 9. Breed comparison and its analysis. Corrected figures in each case.

mating to Mongrels in maximum, but being easily last in persistency. The interest in the Non-Pedigree Shorthorns lies in the fact that they are the cows which are being selected from among the Mongrels, as worthy of grading up for admission into the Herd Book; presumably they are endowed with merits from other points of view, for as milkers they leave much to be desired, being well below the group from which they have been selected both in maximum and persistency.

It appears that, as between breed and breed, maximum and persistency are not associated, for if the correlation between them be

determined (by the method of ranking) it is found to be − 0·086, with these six observations; thus the two characters seem to be genetically distinct. To test their relative importance in determining breed differences the correlations were calculated between the ranking on lactation yield on the one hand, and on maximum and persistency on the other; the correlation coefficients were + 0·714 and + 0·600, respectively, but these of course are extremely rough figures as the observations are so few; on this basis 51 per cent. of the variation in lactation yield is accounted for by variation in maximum, as compared to 36 per cent. by variations in persistency—that is, the maximum is more potent in the ratio of 1·42 to 1.

Herd comparison.

In order to find how much variation there was from herd to herd in lactation yield and in its two components, all herds were taken from which 50 or more lactations had come; there were 20 of them and they furnished the results shown in Table XI. This table gives the means and coefficients of variation of corrected lactation yield, and corrected S.F., and the mean corrected maximum calculated from the other two, for each herd.

Table XI. *Comparison of twenty largest Norfolk herds.*

Herd	Breed of cows	No. of lactations	Corrected lactation yield		Corrected S.F.		Calculated corrected maximum.
			Mean (lb.)	v	Mean	v	Mean (lb.)
H_1.	Red Poll	61	9553	22·57	1·072	13·99	330·1
B.	Friesian	82	8963	19·32	1·031	17·65	322·0
R_2.	Mongrel	80	8731	23·65	1·116	17·21	289·8
B_3.	Friesian	60	8725	23·79	1·037	18·04	311·6
B.	Friesian Cross	85	8603	20·25	1·021	16·06	312·1
G.	Mongrel	104	8563	22·47	1·048	16·32	302·6
K.	Mongrel	116	8470	22·38	1·043	16·54	300·8
Q.	Mongrel	242	7924	23·04	1·070	16·54	274·8
J.	Lincoln Red	94	7894	18·65	0·973	15·72	300·5
T.	Red Poll	69	7591	23·54	1·114	18·22	252·4
P_3.	Mongrel	75	7557	23·59	1·040	19·04	269·1
J_1.	Lincoln Red	95	7445	20·40	0·927	15·10	297·5
Z.	Non-Ped. Shorthorn	63	7274	20·06	0·980	12·96	274·9
C_1.	Lincoln Red	112	7071	26·08	1·003	15·75	261·1
H_3.	Mongrel	155	7031	20·72	0·941	15·41	276·7
Q_3.	Friesian Cross	87	6991	22·85	1·013	16·59	255·6
Y_3.	Ped. Shorthorn	50	6920	23·15	1·050	13·90	244·1
R_1.	Friesian	66	6674	27·15	0·925	18·38	267·2
T_1.	Lincoln Red	109	6635	21·88	0·835	17·36	294·3
M.	Red Poll	171	6516	25·00	0·962	21·10	250·9
	Arithmetic mean of 20 herds		7757	22·53	1·010	16·59	284·4

Herd variation is shown by the means in Table XI and the 20 observations gave the following:

	Mean	Coefficient of variation
Corrected lactation yield	7757 lb.	11·22
Corrected S.F.	1·010	6·63
Calculated corrected maximum	284·4 lb.	8·64

Maximum, therefore, exhibits wider variation from herd to herd than does persistency, in the ratio 8·64 to 6·63, or 1·30 to 1; this accords very closely with the ratio 1·23 to 1 found between lactation and lactation for all the records included (p. 175).

As before, the correlations between the means for the three variables were determined by the ranking methods, with the following results:

S.F. × lactation yield, $r = + 0{\cdot}641$.
Maximum × lactation yield, $r = + 0{\cdot}764$.
S.F. × maximum, $r = + 0{\cdot}081$.

The last is of course quite insignificant, showing that from herd to herd maximum and persistency vary quite independently. The correlation between maximum and lactation yield is again higher than between persistency and lactation yield, and the relative potency in determining herd differences is as $(0{\cdot}764)^2 : (0{\cdot}641)^2$ or as 1·42 : 1; it can only be regarded as a coincidence that this ratio should be exactly the same as the corresponding one found with the breed means, as the values of r, having been derived solely from the ranking of the means, are only rough approximations.

The constancy of the individual.

Fundamentally the justification for milk recording lies in the constancy of the individual cow, for it is no use ascertaining a yield unless it is a reasonably good guide as to what that cow will do in the future—that is, unless each animal has her own peculiar producing ability which is more or less constant throughout her life; the present section aims to find out how constant cows are from lactation to lactation in their yield, and in its two constituents maximum and persistency. That the expression of innate ability may be largely modified by external factors is the basis of this (and similar) work, which has given standardising corrections for four of these factors; there remains one, however, which is not susceptible of similar treatment—the management factor—and so in order to escape that, cows were taken together in groups by herds, on the assumption that management is constant within the several herds;

this assumption is a common one and would appear to be justifiable on general grounds, but in one case given below it will be seen that it did not accord with the facts.

The data included ten herds with more than 25 cows having at least two lactations each:

Herd	Breed	No. of cows with at least two lactations each
K.	Mongrels	35
Q.	,,	39*
H_3.	,,	47
B.	Friesian Crosses	26
Q_3.	,,	38
M.	Red Polls	48
J.	Lincoln Reds	26
C_1.	,,	29
J_1.	,,	32
T_1.	,,	29

* And 38 others with 3 or more lactations each.

As a measure of the constancy from one lactation to the other the intra-class correlation coefficient was used; for each variable the above herds gave ten values of this based on cows with two lactations each, and one based on cows with three lactations each, and these eleven were determined for S.F., maximum, and lactation yield, both raw and corrected, making 66 correlation coefficients in all. Many of the above cows had more than two lactations included in the data, and in these cases, the first two of which full details were known, were taken; in a few cases full details of two lactations were available, except for one D.P.—this was not held to disqualify.

The intra-class correlation coefficients were not obtained from tables in which each pair of lactations was entered twice, but from the standard deviations of all the lactations and of the cow-means; thus in the case of herd K there were 35 cows with 70 lactations; the standard deviation of corrected lactation yield for the 70 lactations was 2042 lb., so that the means of random samples of two should give a standard deviation of $2042/\sqrt{2}$ or 1444 lb.; actually the 35 cow-means gave a standard deviation of 1997 lb., showing that they were more scattered, so that pairs must have tended to be both high or both low—in other words a positive correlation did exist between them; the intra-class correlation in this case is given by $\left(\frac{1997}{1444}\right)^2 - 1$, or $r = +0{\cdot}913$. Where there were three lactations of each cow (second group of herd Q) the formula was

$$r = \tfrac{1}{2}\left\{\left(\frac{\text{Actual } \sigma_m}{\text{Expected } \sigma_m}\right)^2 - 1\right\}.$$

It will be realised that the intra-class coefficient measures a slightly different association from the inter-class one, for the latter is concerned with the efficiency of one observation in giving an estimate of another by means of a regression equation, whilst the former is only concerned with its efficiency as a direct estimate. This is illustrated by the following, which were obtained from the two largest herds:

	Herd H_3.		Herd M.	
	Intra-class r	Inter-class r	Intra-class r	Inter-class r
Raw S.F.	+0·127	+0·195	+0·274	+0·293
„ maximum	+0·518	+0·598	+0·376	+0·456
„ lactation yield	+0·392	+0·506	+0·568	+0·514
Corrected S.F.	+0·296	+0·293	+0·539	+0·524
„ maximum	+0·523	+0·521	+0·322	+0·287
„ lactation yield	+0·505	+0·521	+0·709	+0·559

With one exception the inter-class correlation coefficient is higher with raw figures because the means for the earlier lactations of the various cows differed from the means of the later lactations; the inter-class correlation takes this into account but the intra-class one does not; with corrected figures the earlier and later lactations give the same means and the two correlation coefficients would therefore be expected to be practically identical, and this is fulfilled in every case except one. Thus the intra-class correlation measures the direct association between the observations as they stand, whilst the inter-class correlation measures the closeness of their association along the regression line.

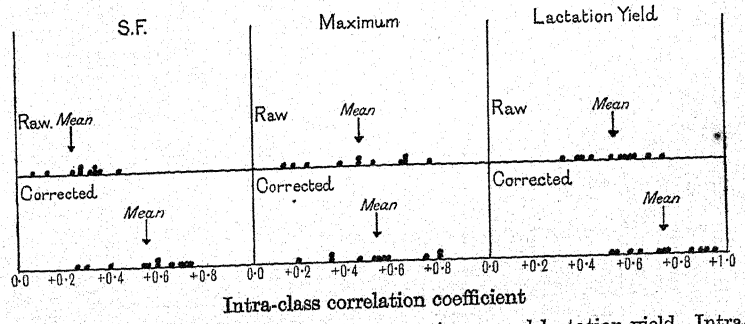


Fig. 10. Constancy of raw and corrected S.F., maximum and lactation yield. Intra-class correlations between successive records of individual cows. Eleven coefficients from ten different herds.

Fig. 10 shows 65 of the 66 intra-class correlation coefficients. The other one (for raw S.F. in herd Q_3) falls off the scale as it is negative, and this was an interesting case, for a negative value would not be expected to occur at all, if cows exhibit any marked constancy; there is little doubt

that the explanation lies in the fact that the general management of this herd was greatly changed during the period covered by the data; this meant that cows had one very poor lactation followed by a comparatively good one, so that the cow-means all tended to be nearer the normal than would be the case with pairs of these lactations drawn at random; in particular the length of S.P. was greatly increased, so that the change in management had its greatest effect on raw S.F.

The following averages of the 11 coefficients in each group of Fig. 10 were obtained:

	Raw	Corrected
S.F.	+0·232	+0·542
Maximum	+0·452	+0·522
Lactation yield	+0·520	+0·731

Raw maximum is more constant throughout the cow's life than raw S.F., but when corrections are made for age, etc., S.F. gives a slightly higher figure; the conclusion must be drawn that cows tend to be equally constant from lactation to lactation in their innate capacity as regards maximum and persistency, but that the expression of the latter is more liable to modification by environmental factors. Lactation yield is more constant than either of the others.

It remains to determine the standard error of estimating a lactation as the same as the previous one; since the intra-class coefficients agree closely with the inter-class coefficients the standard error of the direct estimate was calculated as $\sigma\sqrt{1-r^2}$. The average values of the standard deviations in the 11 herds were:

	Raw	Corrected
S.F.	0·2165	0·1635
Maximum	57·58 lb.	50·52 lb.
Lactation yield	1809 lb.	1668 lb.

Expressing the values of $\sigma\sqrt{1-r^2}$ as percentages of the means, the following were obtained:

	Raw	Corrected
S.F.	21·2	14·3
Maximum	21·3	15·4
Lactation yield	23·5	15·7

Thus the raw S.F. and the raw maximum in one lactation, if used as direct estimates of what the cow will do in her next milking period, are subject to a standard error of 21 per cent.; if, however, corrections are applied the standard errors are reduced to 14 per cent. and 15 per cent. respectively.

Cows show rather less relative constancy in their lactation yields, though the difference is small. If a farmer estimates his cow's yield in

one lactation as the same as in her last his standard error is 23·5 per cent., but this is reduced to less than 16 per cent. if he uses standardising corrections. This last corresponds to a probable error of 10·6 per cent., compared to a figure of 8·2 per cent. found previously (12) from the fluctuations of individuals round their own means; the two errors have rather different meanings, as is evidenced by stating the final result thus: The corrected lactation yield is subject to a probable error of 10·6 per cent. as an estimate of the yield in the next lactation, and to a probable error of 8·2 per cent. as an estimate of the cow's true milking ability as determined from at least three lactations, of which it is one.

The physiological association between maximum and persistency.

There are three aspects of the association of maximum and persistency of some interest, which may be described as the genetic, dairy husbandry, and physiological respectively. From the comparison of the breeds no relation was apparent between maximum and persistency, which would indicate independent inheritance of the two characters, nor was there in comparing herds, which would indicate independent effects on them of good and bad management; these, however, are of the nature of total correlations, and the validity of drawing the obvious conclusions rests on the assumption that there is no correlation physiologically.

The third aspect therefore assumes added importance and an opportunity of studying it was presented by the records of the cows dealt with above in studying constancy. A correlation table was drawn up for these cows between the percentage changes from the earlier to the later lactations of maximum and persistency, using corrected figures; there were 425 observations in all, giving a value of $r = -0{\cdot}372 \pm 0{\cdot}028$.

There is, therefore, a significant negative correlation, showing that an increase in maximum tends to depress persistency and *vice versa*. The absence of any correlation between the two variables from breed to breed would indicate therefore that there is in reality a positive association sufficient to nullify this; this would be expected on the assumption that milk yield is a multiple factor character, for breeding consists in concentrating the favourable genes, some of which will act through the maximum and some through persistency; consequently, in the genesis of the high-producing cow the two characteristics will be developed side by side. As between different herds the nullifying positive association would again be expected, for it is probable that improved conditions will raise both the maximum and the lactation yield, but the latter more, and thus also raise persistency as here defined. The present

negative correlation explains the fact that individual cows are more constant in lactation yield than in either maximum or persistency—for if one is raised the other tends to be depressed, keeping the product relatively steady.

The equation of the regression of percentage change in S.F. (y) on percentage change in maximum (x) was

$$y = -0{\cdot}364\,x.$$

From this it appears that if from one lactation to another the maximum is raised by 20 per cent. the persistency is lowered by 7·28 per cent.; then since lactation yield $= k \times$ maximum $\times$ persistency, it takes the value k (1·20) (92·72) or 111·3 k—*i.e.* it is raised by 11 per cent.

As to the basis of this physiological association between maximum and persistency it is only possible to speculate, but the simplest and most plausible explanation would appear to be a nutritional one; evidence has already been produced that in some cows, where intermediary metabolism is unfavourable to milk production, persistency is largely dependent on storage of food materials, and consequently with them a heavy drain in early lactation might lower the power of sustaining secretion at a high level; this might well account for the negative association of maximum and persistency, but as an alternative view it might be urged that high secretion at first means that the mammary cells are living at a high level, and that this may lead to an increased rate of senility as the lactation progresses; the utility of this, however, waits, as has been pointed out before, on a fuller conception of what the word senility embraces.

SUMMARY.

An attempt has been made to analyse the lactation yield into its two components, maximum yield and persistency; the latter has been defined as the ratio of lactation to maximum yield, and has been measured by a S.F. calculated from $\frac{\text{Lactation yield}}{\text{Maximum yield} \times R}$, where R is the mean ratio for the month of calving concerned.

Whilst, when corrections for external factors are applied, maximum and persistence are equally constant throughout the cow's life, these factors cause wider individual fluctuations with persistency; maximum yield is subject to a more rigid limit and may be largely determined by the area of the mammary gland, but persistency seems to be chiefly a nutritional factor—that is to say, it depends on the success of the mammary gland in competing with the other tissues of the body for the available nutriment. This leads to the view that persistency is higher in

the dairy type of cow; it also appears to be associated with high constitution, for very definite positive selection is apparent with persistency, as compared to marked negative selection for maximum—this is attributed to pathological attrition falling more heavily on cows with high initial yield and low persistency.

The nutritional view of persistency is strengthened by the fact that changes in standardised maximum yield from one lactation to another of the same cow are negatively correlated with changes in standardised persistency; this correlation is obscured between different cows, different breeds and different herds, which would indicate that in breeding for higher production genes favourable to maximum and to persistency are concentrated collaterally, and that good conditions of management raise both the maximum and persistency (*i.e.* raise the lactation yield in a greater proportion than the maximum yield).

Persistency is, of course, largely affected by the length of the service period, but maximum only slightly. The age variation in maximum is an exaggerated form of the age variation in lactation yield; persistency, on the other hand, declines with age, rapidly from the first to the second lactation and after that at a constant and slower rate.

The effect of the length of the dry period appears as a dual one; a rest of 40 days is necessary for the full development of the maximum yield, and any further period is reflected solely in increased persistency. From this the conclusion has been drawn that inaction for a period of 40 days, and no more, is necessary for the full regeneration and development of the mammary gland, and that the benefit of further rest lies in its allowing of the accumulation of food reserves; these latter have an important influence in keeping the yield up as the lactation progresses in the case of cows that are naturally poor in persistence, whilst they have little or no influence on those at the other extreme, in which, it has been concluded, intermediary metabolism is favourable to milk production.

A system of standardising lactation records which would vary the correction factors with the persistency level of the cow, would be too onerous in operation to be of any practical value, but attention is drawn to the fact that any simple system must be in the nature of a compromise, and correspondingly inaccurate. With persistent cows the effect of the month of calving on lactation yield is very much toned down as compared to non-persistent cows, and the latter show a much greater age variation —a rise of 40 per cent. from the 1st to the 6th lactation, as compared to 25 per cent. Also it follows from the above remarks that the form of the

D.P. effect on lactation yield must differ markedly between persistent and non-persistent cows, and this was found very definitely to be the case.

The writer has great pleasure in acknowledging his deep indebtedness to Mr J. Hammond, M.A., for his constant interest and helpful and stimulating comments.

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(*Received December 14th*, 1929.)

EXPERIMENTS ON THE TREATMENT OF PARASITIC GASTRITIS IN SHEEP AND LAMBS.

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During the past few years the question of the treatment of parasitic gastritis in the sheep and the lamb has occupied the attention of several investigators, and the effect of a number of drugs has been tried on infested sheep kept under experimental conditions. According to some authors, certain of these drugs have given quite satisfactory and reliable results, especially against *Haemonchus contortus*, but these results have been contested by other observers.

Hall(1) found that a 1 per cent. solution of copper sulphate (10–15 mils.) removed the majority of *H. contortus* from two heavily infested sheep, but had no marked effect on nodular worms. The treated sheep passed 120, 240 and 314 stomach worms, respectively, and showed on post-mortem examination, 0, 49 and 3 parasites in the abomasum. The control sheep showed over 4000 and 6000 stomach parasites, respectively. The small number of worms which was recovered from the faeces (less than 10 per cent.) is adjudged to be dependent, in all probability, on the rapid disintegration of dead parasites in the stomach. Copper sulphate failed to remove hookworms in the sheep. Chloroform and gasoline compared very unfavourably with copper sulphate, and oil of chenopodium was not very promising. Petroleum benzine was somewhat inferior to copper sulphate against *Haemonchus*, but was fairly efficacious against *Bunostomum*, removing two-thirds of these parasites. In a later paper, Hall(2) mentions that at the experimental station of the B.A.I. the routine use of 1 per cent. copper sulphate every month proved an astonishingly satisfactory method of control of the stomach worm in sheep. The efficacy of repeated doses of copper sulphate in the control of stomach worms is also mentioned by Curtice(3) and Veglia(4).

Veglia(4), in an important paper on the treatment of *H. contortus*, gives details of the efficacy of several drugs. The sheep used in the experiments were artificially infected with larvae of *H. contortus* either by direct intrarumenal injection through a syringe or by feeding the larvae. The efficiency of the drugs tested was checked by post-mortem examinations of both the treated and control sheep in each experiment. The general conclusions reached on the experiments with a single drug

were that they were never completely successful against *Haemonchus*, even when administered in quantities approaching the fatal dose for one sheep. For example, picric acid, thymol, beta naphthol, lysol, gasoline and creosote were all ineffective to a varying degree. The most satisfactory results were obtained with a mixture of copper sulphate and sodium arsenite or copper sulphate and Cooper's dip. Copper sulphate alone in doses of 650 mg. on three successive days was unsuccessful and sodium arsenite alone in doses confined to the limits of safety was not completely effective. 500 mg. each of Cooper's dip and copper sulphate for two days in succession removed all the *Haemonchus* from sheep in the last stages of a very heavy infestation. Copper sulphate and sodium arsenite were equally effective. The doses of the two drugs recommended for sheep of varying ages were as follows: adult sheep, copper sulphate 400 mg. and sodium arsenite 100 mg.; lambs 6–10 months, 300 mg. and 75 mg.; lambs 4–6 months, 200 mg. and 50 mg.; lambs 2–4 months, 140 mg. and 35 mg. The drugs were most effective in powder form, but owing to the danger of a portion of the dose failing to reach the stomach, the liquid form was recommended. The addition of hydrochloric acid ensured complete solution of the drugs.

The percentage efficiency of the drugs was as follows: copper sulphate and sodium arsenite 100 per cent., gasoline 70 per cent., beta naphthol 55 per cent., thymol 50 per cent., creosote 20 per cent., lysol 10 per cent.

Sheather (5) carried out some experiments on naturally infested sheep in which the degree of infestation was estimated by egg counts on the faeces. The efficacy of the drugs used was checked by repeated egg counts, and on the results of the post-mortem examination of treated and untreated animals. His experiments tended to support the conclusions of Veglia on the value of a solution of sodium arsenite and copper sulphate and of copper sulphate alone against *Haemonchus*. These drugs were ineffective against *Ostertagia circumcinta*, *O. trifurcata*, *Trichostrongylus extenuatus*, *T. instabilis* and *T. vitrinus*. Bleaching powder, sodium bisulphite with hydrochloric acid, carbon tetrachloride, derris root, copper oleate, oil of chenopodium, thymol in turpentine, and lime and sulphur wash were ineffective against any species encountered.

The addition of mustard to the solution of copper sulphate is said to enhance its efficiency, and this mixture has been given an extensive trial in Australia. Seddon and Ross (6) state that it is unquestionably the safest method for the sheep. They are of the opinion that both copper sulphate and mustard, and carbon tetrachloride are good methods of treatment with an efficiency of 75 per cent. and upwards. The doses

recommended are 5 c.c. of carbon tetrachloride and 4·86 gm. of copper sulphate and of mustard each for lambs of 30–40 lb. Wright(7) found that mustard alone exhibited very small powers against *H. contortus* and *Monodontus trigonocephalus*. In another experiment a 1 per cent. copper sulphate solution with mustard left 700 *H. contortus* surviving in the stomach. Branford(8) recommends the copper sulphate and mustard mixture for the treatment of *Haemonchus*.

In more recent communications, Hall and Shillinger(9) put forward the claim that carbon tetrachloride is superior to copper sulphate. In one experiment, four lambs were dosed with 12, 18, 24 and 48 c.c. of carbon tetrachloride, respectively, followed by 2 oz. of castor oil. *H. contortus* was recovered in small numbers from the faeces. On post-mortem examination, all were free from stomach worms (presumably *Haemonchus*). The claim is made for an efficiency of 100 per cent. In a second experiment, two heavily infested lambs received 15 and 30 c.c. of oil of chenopodium, respectively. Three stomach worms were collected in the faeces of one and none in the other, whilst on post-mortem examination no worms were found in the stomachs of either lamb. Failure to obtain more worms in the faeces was ascribed to digestion of the parasites in the stomach. A control animal was killed and showed 612 worms. It is stated that carbon tetrachloride removed 82 per cent. of 801 small *Trichostrongylus* (*Nematodirus*, *Cooperia*, *Ostertagia* and *Trichostrongylus*). In a later paper, Hall and Shillinger(10) recommend the addition of magnesium sulphate to carbon tetrachloride with a view to impeding the absorption of the latter drug. In one experiment, a dose of 10 c.c. of carbon tetrachloride with 4 oz. of magnesium sulphate removed all of 174 trichostrongyles. Schillingman(11, 12) advocates the use of tetrachlorethylene in doses of 5 c.c. in capsules, stating that previous research has demonstrated the effectiveness of this drug against stomach worms.

PRESENT INVESTIGATION.

The experiments which are recorded in this paper were undertaken with the object of testing the anthelmintic properties of various drugs on sheep naturally infested with stomach worms. It is generally recognised that, in order to be of practical value in the field, any preparation used for the treatment of sheep must be reasonably inexpensive, safe in use, easily administered and capable of producing its effects in one or two doses, or given mixed in the food over a period, and these points have not been overlooked.

The sheep used in the experiments were kept in separate pens, and the faeces were collected at frequent intervals. The number of eggs per gm. of faeces, as estimated by the sugar flotation method devised by Sheather(13), gave an approximate idea of the extent of the worm infestation. It must be pointed out, however, that an examination of this nature only reveals the fact that the animal is or is not a host of parasites in its digestive tract. It is not possible, from an examination of the eggs, to state what species of worms are present, or where they are located, since, with the exception of two which are easily recognised, viz. *Nematodirus*, the eggs of which are very large, and *Strongyloides*, which has very small eggs usually containing embryos, the eggs of the various worms commonly found in the stomach and intestines of the sheep are so alike in size and appearance that their identification is a matter of considerable difficulty. An attempt was made to estimate the species of worms with which some of the sheep were infested by measuring a large number of eggs and comparing them with those of the commoner sheep worms. Unfortunately, although many thousands of eggs were measured, no useful results were obtained. The measurements agreed, however, with those of the eggs of the commoner parasites of sheep, as recorded by Hall in America(14). It was also noted that the size of worm eggs tended to vary within well-defined limits under certain conditions. For example, the eggs from one species of worm from the stomach of one sheep tended to vary in size from those of the same species from another sheep, although examined under the same conditions.

Another point to be considered, and one that may explain the irregularity in many of the egg counts of individual sheep, is that, in all probability, roughly the same number of eggs are passed daily by any given infested sheep over a more or less short period. In those cases where for some reason the animal loses its appetite, or the feed is changed from a bulky to a more concentrated ration, the number of eggs passed per gramme of faeces at once augments considerably, varying with the amount of faeces which are passed per diem. It must also be recognised that the state of the faeces may also affect the egg count. Since in the following experiments, the egg count was made on a given weight of faeces, the total amount of dry matter in any given sample varied considerably from day to day, depending on whether the faeces were in a dry or a wet condition. Undoubtedly, it would have been better to have taken the total weight of faeces passed per day and worked out the result as eggs per sheep per day, rather than to show the results as eggs per gm. of faeces. In consequence, it will be

recognised that statistics based on the number of eggs passed per gm. of faeces may be very inexact. Nevertheless, it is fair to assume that where the egg counts dropped considerably in numbers for several consecutive counts without any change in the food eaten, the number of worms had been lowered.

The sheep used were of various ages and breeds, and had been kept in the fields of the Department for some time prior to their use. Some were very heavily infested, whilst in others the number of parasites was small. In heavily infested sheep, post-mortem examination almost invariably revealed the presence of *O. circumcinta* and *T. extenuatus*. *H. contortus*, although not so frequent, was sometimes found in very great numbers. In some cases, especially in young lambs in which *Nematodirus* was the most common parasite, the number of worms in the small intestine exceeded by far those of the abomasum.

Throughout these experiments, where *O. circumcinta* is mentioned, it should be taken as recording the numbers of that worm together with a small percentage of *O. trifurcata*. Since the females of the two species are indistinguishable from one another and since, as far as is known, the life histories are identical, it seemed unnecessary to give the numbers of the males of each species separately. In the small intestines *T. vetrinus* was commonly found together with a smaller number of *T. instablis*. Of the *Cooperia*, *C. Curticii* was by far the most numerous. The eggs of *Strongyloides papillosus* were almost invariably found, sometimes in large numbers, but they have not been included in the records of egg counts.

Several sheep were given capsules immediately before they were killed and, in each case, the capsule was recovered from the rumen or reticulum. Similarly, other sheep were given fluid drenches of an oily nature and, on each occasion, much of the oil was found in the abomasum.

A number of different drugs were tested. Some were given in solution either in capsules or by the stomach tube, some were given dry in hard gelatin capsules and others were mixed with the daily food. Except in the latter case, the sheep were always fasted for at least 24 hours and water was also withheld for an equal time. They were not given food or water for some hours after dosing.

It can be stated that none of the drugs which were tested had any appreciable effect on any of the parasites other than *H. contortus* with the possible exception of petroleum ether which seems to be worthy of a further trial. Since *Haemonchus* was not present in every case, a considerable drop in the egg count following the administration of any

of the drugs used would seem to point to its presence in the animal and to the reduction being due to the destruction of this parasite.

Experiment No. 1.

Drug used: copper sulphate (4 parts) and sodium arsenite (1 part).
Dose given: 4 gm. of copper sulphate; 1 gm. of sodium arsenite.
Method of administration: dry in a gelatin capsule.

Lamb 1. 1 year old—fair condition.

Days before 1st dose	2	1					
Eggs per gm. of faeces	250	600					
Days after 1st dose		1	2	3	4	5	6
Eggs per gm. of faeces		125	782	95	1000	150	50

2nd dose given 7 days after 1st dose.

Days after 2nd dose	2	4	6	8	10	12	14
Eggs per gm. of faeces	125	50	36	183	100	78	90

3rd dose given 15 days after 2nd dose.

Days after 3rd dose	1	3	5	7	8	11	14	16
Eggs per gm. of faeces	205	400	300	330	450	388	187	169
Days after 3rd dose	18	21	23	27	31			
*Eggs per gm. of faeces	194	206	121	330	550			

Result.

The reduction following the 1st dose was probably due to destruction of the *H. contortus*. Post-mortem examination of other lambs from the batch from which this lamb was taken showed heavy infestation of *Haemonchus* and *Ostertagia* with a smaller number of *Trichostrongylus* in the fourth stomach.

Lamb 3. Very poor—dosed with 0·375 gm. of copper sulphate and sodium arsenite mixture 4 : 1.

Egg count prior to dosing—3000				
Days after 1st dose	2	3	4	5
Eggs per gm. faeces	5000	2500	2000	3100

2nd dose 0·375 gm., given 8 days after 1st dose.

Died 2 days after 2nd dose.

Post-mortem examination showed large numbers of worms in the fourth stomach, chiefly *H. contortus*. There was also a heavy infestation with coccidia.

Experiment No. 2.

Drug used: copper sulphate.
Dose given: 1 gm.
Method of administration: dry powder in hard gelatin capsule.

Lamb V. Cross-bred lamb in fair condition.

Days before 1st dose	1	2	3					
Eggs per gm. faeces	1250	1000	100					
Days after 1st dose	1	2	3	4	5	6		
Eggs per gm. faeces	2600	1000	420	250	125	735		

2nd dose given 8 days after 1st dose.

Days after 2nd dose	1	4	6	8	10	12	14	
Eggs per gm. faeces	42	126	12	200	46	30	70	

3rd dose given 15 days after 2nd dose.

Days after 3rd dose	2	4	6	8	11	13	16	18
Eggs per gm. faeces	123	450	436	67	222	141	88	43

Result.

Considerable reduction in number of eggs after doses 1 and 2. Probably due to destruction of *Haemonchus*.

Ewe 10. Cheviot, in very poor condition.

Days before 1st dose	1	2	3			
Eggs per gm. faeces	200	300	250			
Days after 1st dose	1	2	3	4	5	6
Eggs per gm. faeces	500	100	78	365	135	135

2nd dose given 7 days after 1st dose.

The ewe died on the second day after the 2nd dose. The post-mortem examination showed many *O. circumcinta* and a few *T. extenuatus* in the abomasum. The large intestine contained many *Oesophagostomum dentatum*. The liver was affected with fatty degeneration, and the lungs showed extensive areas of verminous pneumonia. No *H. contortus* were found.

EXPERIMENT No. 3.

Drug used: copper sulphate.

Dose given: 1 gm.

Method of administration: 1 per cent. solution in water given by stomach tube.

Ewe 2. 2 years, poor condition.

Days before 1st dose	1	2	3				
Eggs per gm. faeces	200	300	250				
Days after 1st dose	1	2	4	5	6		
Eggs per gm. faeces	50	200	250	120	70		

2nd dose given 7 days after 1st dose.

Days after 2nd dose	1	4	5	8	10	12	14
Eggs per gm. faeces	20	9	25	75	35	23	82

3rd dose given 15 days after 2nd dose.

Days after 3rd dose	4	6	8	11	13	16	18
Eggs per gm. faeces	90	200	80	100	18	6	12

33 days after 3rd dose a 4th and increased dose was given, viz. 150 c.c. of 1 per cent. solution.

Days before 4th dose	7	3	1				
Eggs per gm. faeces	118	46	50				
Days after 4th dose	1	2	4	6	8	10	12
Eggs per gm. faeces	152	54	40	75	46	85	105

Lamb 12. Small and in poor condition.

Days before 1st dose	2	1
Eggs per gm. faeces	500	450

1st dose 85 c.c. 1 per cent. solution.

Days after 1st dose	2	3	4	5	6
Eggs per gm. faeces	98	200	500	300	750

2nd dose 60 c.c. 1 per cent. solution, 7 days after 1st dose.

Days after 2nd dose	4	6	8	10	12	14
Eggs per gm. faeces	57	31	90	72	40	128

3rd dose 70 c.c. 1 per cent. solution, given 15 days after 2nd dose.

Days after 3rd dose	4	6	8	11	13	16	18
Eggs per gm. faeces	70	460	66	117	119	119	72

39 days after 3rd dose a 4th dose of 110 c.c. was given.

Days before 4th dose	9	7	3	1			
Eggs per gm. faeces	184	90	53	179			
Days after 4th dose	1	2	4	6	8	10	12
Eggs per gm. faeces	104	24	93	82	40	92	75

Lamb 21. Small and in very poor condition.

This lamb was given 100 c.c. of a 1 per cent. copper sulphate solution by stomach tube. Killed 4 hours after dosing.

Very large numbers of *H. contortus* and *Ostertagia circumcinta*, and a few *T. extenuatus* were found in the contents of the abomasum which was examined as soon as possible after death. About 75 per cent. of the *Haemonchus* and 25 per cent. of the *Ostertagia* showed no movement and were apparently dead.

Result.

Copper sulphate in 1 per cent. solution and doses of 100 c.c. for sheep causes considerable reduction in the number of eggs passed in most cases, the reduction being most probably due to destruction of *H. contortus*.

Experiment No. 4.

Drugs used: turpentine, 5 c.c.; chloroform, 75 c.c.; linseed oil, 75 c.c.

Dose given: 35 c.c. of the above mixture.

Method of administration: in liquid by stomach tube.

Lamb 9.

Days before 1st dose	1								
Eggs per gm. faeces	100								
Days after 1st dose	1	2	3	4	5	6	7	8	9
Eggs per gm. faeces	137	1000	600	180	3500	2800	100	163	1000

2nd dose 60 c.c. of mixture, 12 days after 1st dose.

Days after 2nd dose	2	5	7	9	11	13	14
Eggs per gm. faeces	500	1600	355	750	500	300	1000

Killed on 15th day after 2nd dose.

The abomasum contained a very large number of living worms including *H. contortus, Ostertagia circumcinta* and *T. extenuatus*. In the small intestine many *Cooperia Curticii* and *Nematodirus fillicollis* were found. The caecum and colon were devoid of parasites.

Result.

No reduction in egg count. The mixture does not appear to possess any vermicidal action.

EXPERIMENT No. 5.

Drug used: carbon tetrachloride.

Dose given: 12 c.c.

Method of administration: in gelatin capsules.

Cheviot Ewe 13.

Days before 1st dose	5	4	3	2	1					
Eggs per gm. faeces	900	100	75	200	400					
Days after 1st dose	1	2	5	7	10	12	14	16	18	20
Eggs per gm. faeces	10	14	50	20	9	1	21	4	6	65

2nd dose 12 c.c. given 21 days after 1st dose.

Days after 2nd dose	1	3	5	7	9	11	14	16	18	20
Eggs per gm. faeces	150	48	60	40	116	45	25	73	50	41

Cheviot Lamb 620.

Days before 1st dose	7	5	3	1						
Eggs per gm. faeces	204	100	70	320						
Days after 1st dose	1	3	5	7	9	11	14	16	18	20
Eggs per gm. faeces	1650	1560	1100	101	205	111	73	110	81	119

2nd dose given 22 days after 1st dose.

Days after 2nd dose	1	2	4	6	8	12	14	16	19
Eggs per gm. faeces	69	117	21	41	36	35	63	83	98

Cheviot Lamb 626.

Days before 1st dose	5	3	1							
Eggs per gm. faeces	210	151	400							
Days after 1st dose	1	3	5	7	9	11	14	16	18	20
Eggs per gm. faeces	1500	1000	600	210	237	220	80	241	172	142

22 days after 1st dose a 2nd dose, 13 c.c., was given.

Days after 2nd dose	1	2	4	6	8	12	14	20
Eggs per gm. faeces	530	630	121	192	150	129	185	139

Lambs 620 and 626 were off their feed for several days after each dose, with a resulting decrease in the amount of faeces passed. This might account for the increased egg count.

Result.

Carbon tetrachloride in 12 c.c. dose does considerably reduce the numbers of eggs passed.

EXPERIMENT No. 6.

Drug used: chloramine tabloids (8·75925).
Dose varying.
Method of administration: in capsules.

Lamb 4. Previously used for Exp. 1.

Days before 1st dose	6	2	0	(dose 2 tabloids)			
Eggs per gm. faeces	330	550	559				
Days after 1st dose	1	3	5	7	9	11	13
Eggs per gm. faeces	404	193	244	147	100	167	100

2nd dose of 3 tabloids, given 16 days after 1st dose.

Days after 2nd dose	1	3	5	8
Eggs per gm. faeces	185	49	100	115

Lamb 12. Given 1 dose of 1 tabloid in gelatin capsule. No appreciable effect on the egg count.

Ewe 7. Given 4 tabloids. No appreciable effect on the egg count.

Result.

No reduction in the number of eggs.

EXPERIMENT No. 7.

Drug used: sodium pentasulphide.
Dose used: varying.
Method of administration: in gelatin capsules.

Cheviot Ewe Lamb 2.

Days before 1st dose	0	(dose 2 c.c.)						
Eggs per gm. faeces	1600							
Days after 1st dose	2	3	5	7	8	9	11	14	15
Eggs per gm. faeces	835	1700	1320	1050	1585	1509	1334	2000	1500

Faeces coated with mucus.

Dorset Ewe N. Eggs per gm. faeces for some days averaging 50 before 1st dose of 5 c.c.

Days after 1st dose	2	5	6	8	10	12	14	16
Eggs per gm. faeces	103	12	65	58	35	108	29	87

Lamb V. Average number of eggs per gm. faeces for some days before 1st dose—500.

1st dose 5·0 c.c. in capsule.

Lamb killed 32 hours after this dose. The abomasum contained large numbers of actively moving worms, chiefly *Ostertagia*, with a smaller number of *Trichostrongylus extenuatus*.

The action of the drug on the parasites *in vitro* was tested in the following manner: The stomach was kept warm and the worms removed to Petri dishes which were kept floating on warm water. The worms were placed into varying dilutions of sodium pentasulphide acidified with HCl.

In 1/5000 solution of sodium pentasulphide in normal saline, active for $1\frac{1}{4}$ hours.

In 1/5000 solution of sodium pentasulphide with HCl added, active for $1\frac{1}{4}$ hours.

In 1/4000 solution of sodium pentasulphide with HCl added, active for $1\frac{1}{4}$ hours.

In 1/500 solution of sodium pentasulphide with HCl added, are dead in $1\frac{1}{4}$ hours. Some movement after 45 minutes.

Cheviot Ewe 622. Given 2 c.c. sodium pentasulphide in 150 c.c. of water. Killed $3\frac{1}{2}$ hours afterwards.

Post-mortem examination showed many actively moving worms in the fourth stomach, the majority being *H. contortus*. Many of these were attached to the stomach walls.

Result.

Sodium pentasulphide in the doses used would seem to be ineffective.

EXPERIMENT No. 8.

Drug used: proprietary arsenic tabloids.

Dose given: 2 tabloids.

Method of administration: given as pills.

Cheviot Ewe Lamb 2. Before 1st dose average number of eggs per gm. faeces—500.

Days after 1st dose	1	2	4	6	8
Eggs per gm. faeces	542	600	513	297	549

Lamb V. Before dosing average number of eggs per gm. faeces—500.

Days after 1st dose	1	2	4	6	8
Eggs per gm. faeces	952	637	440	400	700

Dorset Ewe N. Average number of eggs per gm. faeces before dosing—350.

Days after 1st dose	1	3	4	5	6	8	10
Eggs per gm. faeces	386	156	14	150	52	45	100

Result.

The drug appears to present no advantage over copper sulphate. No effect was noted in Cases 1 and 2 and only a partial effect in Case 3.

EXPERIMENT No. 9.

Drug used: proprietary copper and arsenic mixture.
Dose given: that prescribed by makers and double that dose.
Method of administration: in solution by stomach tube.

Lamb G.L. In poor condition.

Days before 1st dose	4	1
Eggs per gm. faeces	190	266

Dose as prescribed by makers.

Days after 1st dose	1	2	4	6	8	10	13
Eggs per gm. faeces	546	250	367	275	250	115	213

Double dose given 16 days after 1st dose.

Days after 2nd dose	1	2	5	7	9
Eggs per gm. faeces	250	156	180	280	226

Ewe 2. Yearling cheviot.

Days before 1st dose	4	1
Eggs per gm. faeces	1500	2100

1st dose, as prescribed by makers.

Days after 1st dose	1	5	6	8	10	13
Eggs per gm. faeces	1500	1002	1265	1500	2650	1550

Double dose given 16 days after 1st dose.

Days after 2nd dose	1	2	5	7	9
Eggs per gm. faeces	400	150	260	290	425

Results.

In Lamb, G.L., the fact that there was no reduction in the egg count points to the absence of *H. contortus*. In the case of Ewe 2 the large drop following the 2nd dose would seem to indicate that a considerable number of worms, probably *Haemonchus*, were killed.

EXPERIMENT No. 10.

Drugs used: copper sulphate (8 parts); arsenic (1 part); muriatic acid (5 parts); water (60 parts).

Dose given: 8·5 c.c. of mixture.

Method of administration: in solution by stomach tube.

Lamb V. Cheviot.

Days before 1st dose	3	2	1			
Eggs per gm. faeces	1700	1500	1200			
Days after 1st dose	1	2	4	7	12	15
Eggs per gm. faeces	600	600	1050	1050	450	1100

Lamb 0.

Days before 1st dose	3	1				
Eggs per gm. faeces	1500	1400				
Days after 1st dose	1	3	6	8	10	12
Eggs per gm. faeces	1200	1500	1200	1000	1150	1150

Lamb 1. Cheviot.

Days before 1st dose	3	1				
Eggs per gm. faeces	1050	1200				
Days after 1st dose	1	3	6	8	10	12
Eggs per gm. faeces	1200	1700	1400	1300	1000	1250

Result.

No appreciable drop in the egg count.

EXPERIMENT No. 11.

Drug used: monsol.

Various doses were used on several sheep but no appreciable drop in the egg count was obtained in any of the experiments. 2 c.c. doses in capsules were given at 7-day intervals to one lamb for several weeks but with no useful results.

Result.

Not effective as an anthelmintic in any of the doses given.

EXPERIMENT No. 12.

Drug used: Gow's tobacco powder containing 33 per cent. sulphur.

Dose given: 4 gm. per diem.

Method of administration: dry powder mixed daily in the morning feed.

Lambs 4, 5.

Days before 1st dose	6	4	2	
Eggs per gm. faeces	1050	850	1050	(Lamb 4)
Ditto.	750	860	1150	(Lamb 5)

Daily doses in morning feed for 14 days.

Days after commencement	2	4	7	10	14	last day of dosing		
Lamb 4. Eggs per gm. faeces	1060	1120	980	750	300			
Lamb 5. Ditto.	1210	1450	650	500	—			
Days after last dose	1	2	4	8	10	12	15	19
Lamb 4. Eggs per gm. faeces	275	370	245	560	500	350	500	300
Lamb 5. Ditto.	280	1060	400	150	450	190	350	575
Days after last dose	21	23						
Lamb 4. Eggs per gm. faeces	40	140						
Lamb 5. Ditto.	248	425						

Result.

A very marked drop in the number of eggs per gm. of faeces. The lambs improved in condition considerably. The dose of tobacco powder was well tolerated and the food all eaten.

Experiment No. 13.

Drug used: powdered tobacco.

Dose given: 2 gm. and 4 gm.

Method of administration: dry powder mixed with morning feed daily.

Lambs 0, 1. Daily doses of 2 gm. in morning feed.

Days before commencing daily dosing	6	4	1			
Lamb 0. Eggs per gm. faeces	975	1050	975			
Lamb 1. Ditto.	980	620	1250			
Days of daily dosing	1	3	6	8	10	13
Lamb 0. Eggs per gm. faeces	820	800	654	825	746	712
Lamb 1. Ditto.	1272	1000	744	1110	1260	1484

Dose increased to 4 gm. per day in morning feed.

Days of increased dose	2	6	8	10	14
Lamb 0. Eggs per gm. faeces	800	1072	650	334	1194
Lamb 1. Ditto.	1492	2342	4200	2206	1778
Days after 28th dose	2	5	7	9	
Lamb 0. Eggs per gm. faeces	306	908	1096	1372	
Lamb 1. Ditto.	1850	1200	808	2264	

Lamb 1 refused food mixed with tobacco on the 5th and 6th day after the increase of dose. This may account for the rapid increase in the egg count.

Lamb 1 was killed on the 40th day after the 1st dose. The post-mortem examination showed many *Ostertagia* and *Trichostrongylus* in

the fourth stomach. The small intestine contained very many *Trichostrongylus* (*vetrinus* and *instablis*).

Four *Truchuris ovis* were found in the caecum and colon. *H. contortus* was absent.

Result.

The drug had no appreciable effect on the egg count even in a dose of 4 gm. daily. This dose was well tolerated by Lamb 0, but Lamb 1 was affected after 4 days.

EXPERIMENT No. 14.

Drug used: sublimed sulphur.

Dose given: 5 gm.

Lamb 0 *c*. After receiving a dose of tobacco daily in the food for 28 days was given 5 gm. sulphur sub. daily in morning feed.

Days before 1st dose	4	2			
Eggs per gm. faeces	1096	1372			
Days of daily dosing	1	3	6	8	10
Eggs per gm. faeces	1552	1116	944	1094	800
Days after final dose	3	20			
Eggs per gm. faeces	1640	1550			

Result.

No appreciable drop in the egg count.

EXPERIMENT No. 15.

Drugs used: tobacco powder and sulphur.

Dose given: tobacco powder $3\frac{1}{3}$ gm.; sulphur sublimed $1\frac{2}{3}$ gm.

Method of administration: dry powder mixed with the morning feed.

Lambs 0, 7. Given daily doses for 14 days.

Lamb 0 died shortly after the termination of the experiment. Post-mortem examination showed very many worms in the 4th stomach with the following percentage: *H. contortus*, 80 per cent.; *Ostertagia circumcinta*, 10 per cent.; *T. extenuatus*, 10 per cent.

Lamb 0 had been used for the sulphur alone experiment.

Result.

No appreciable lowering of the numbers of eggs passed per gm. of faeces. This dose was eaten readily and no ill-effects were noticed.

Experiment No. 16.

Drugs used: copper sulphate and sodium chloride.
Dose given: copper sulphate 0·25 gm., sodium chloride 4 gm.
Method of administration: mixed with morning feed daily for 14 days.

Lamb 7.

Days before 1st dose	5	2						
Eggs per gm. faeces	408	545						
Days of daily dosing	1	3	6	8	10	14		
Eggs per gm. faeces	476	470	794	606	302	782		
Days after last dose	2	4	8	11	14	16	18	23
Eggs per gm. faeces	314	300	410	332	446	454	292	446

This lamb was kept under observation and by the 100th day after commencement of treatment the egg count had gone up to 3600—showing a heavy infestation. It died shortly afterwards and the post-mortem examination showed the following worms present: In the fourth stomach—*H. contortus*, 80 per cent.; *Ostertagia circumcinta*, 9 per cent.; *T. extenuatus*, 11 per cent. In the small intestine—*T. instablis*, 50 per cent.; *Bunostomum trigonocephalum*, 33 per cent.; *Trichuris*, 13 per cent.; *Oesophagostomum venulosum*, 53 per cent.

Result.

Very slight drop in the number of eggs.

Experiment No. 17.

Drug used: B.P. Petroleum Ether.
Dose given: 10 c.c.
Method of administration: by stomach tube.

Lamb 20.

Days before 1st dose	4	1				
Eggs per gm. faeces	2020	1140				
Days after 1st dose	3	7	10	12	14	21
Eggs per gm. faeces	944	880	900	686	400	190

Another lamb with a very high egg count but in extremely weak condition was also given a 10 c.c. dose. It died 4 days afterwards. Unfortunately, the post-mortem examination could not be made immediately after death, and therefore it was not possible to say if the worms were living at the time when the animal died.

Many worms, chiefly *H. contortus*, were found in the fourth stomach.

Result.

An appreciable drop in the number of eggs passed per gm. of faeces, and an improvement in the condition of the lamb.

Note.

Daubney (personal communication) states that in his experience this drug will reduce the number of *Ostertagia*.

EXPERIMENT No. 18.

Drug used: tetrachlorethylene (Nema).
Dose given: 5 c.c.
Method of administration: in capsules.

Lamb 8.

Days before 1st dose	7	0						
Eggs per gm. faeces	706	774						
Days after 1st dose	4	7	10	12	19	20	22	26
Eggs per gm. faeces	100	130	106	54	86	64	64	42
Days before 1st dose	6	3	1					
Lamb 4. Eggs per gm. faeces	314	844	336					
Lamb 5. Ditto.	904	3100	1300					
Lamb 9. Ditto.	2186	2800	3500					
Days after 1st dose	2	3	4	6	12	13	14	15
Lamb 4. Eggs per gm. faeces	556	684	392	660	512	502	684	484
Lamb 5. Ditto.	1074	876	1678	2200	1982	924	1116	1388
Lamb 9. Ditto.	800	220	574	462	494	348	484	646

2nd dose of 5 c.c. given to Lambs 5 and 9 on 18th day after 1st dose.

Days after 2nd dose	0	4	5	7	10	18
Lamb 5. Eggs per gm. faeces	724	82	44	82	80	44
Lamb 9. Ditto.	520	78	98	92	88	256

3rd dose given to Lamb 9, 19 days after 2nd dose.

Days after 3rd dose	2	3	5	7	
Eggs per gm. faeces	280	104	78	58	killed on 7th day after 3rd dose

Aged sheep. Dose 5·0 c.c. Nema in capsule. Ewes Nos. 0, 10, 11.

Days before 1st dose	9	1					
No. 0. Eggs per gm. faeces	550	260					
No. 10. Ditto.	850	1320					
No. 11. Ditto.	285	600					
Days after 1st dose	2	4	5	7	9	22	26
No. 0. Eggs per gm. faeces	480	758	—	—	270	80	152
No. 10. Ditto.	—	1950	1800	680	—	—	—
No. 11. Ditto.	—	—	—	—	96	218	40

These sheep were very constipated after the treatment, and were also kept in pens with dry peat moss litter. On some occasions, no droppings could be found, hence no egg counts could be made.

Post-mortem examination of those killed are recorded below and a list of the worms found is given in a table at the conclusion of this experiment.

Lamb 9 was killed on the 7th day after the 3rd dose of Nema. A very small number of worms was found in the fourth stomach. A slight inflammation of the small intestine was noted.

Post-mortem examination of Ewe 10, killed 7 days after 5 c.c. dose of Nema, showed a large number of living worms in the fourth stomach (*H. contortus* and *Ostertagia circumcinta*).

The small intestine contained 26 *Nematodirus filicollis* and 30 *Bunostomum trigonocephalum*. There was well-marked, but slight, inflammation of the mucous membrane throughout the small gut.

Nos. 8, 4, 5, 0, 11 were subsequently given one dose of 5 c.c. Nema and killed after a lapse of 7 days.

No. 8 was killed 165 days after the dose of Nema on 23. x. 28. Carcass in prime condition. The egg count had in the meantime gone up again a little and a few worms were found on post-mortem. None of the internal organs showed any apparent abnormality.

No. 4 was killed 143 days after the dose of Nema on 24. x. 28. Carcass fat. The egg count had not increased, and with the exception of *Bunostomum trigonocephalum* in the small intestine very few worms were found on post-mortem examination. All internal organs were apparently normal.

No. 5 was killed on 25. x. 26, 105 days after last dose of Nema, in good condition. All internal organs apparently normal. Very few worms were found on post-mortem examination, *Cooperia Curticii* being the most numerous.

No. 0 was killed 83 days after the dose of Nema on 22. x. 28, in prime condition. All internal organs healthy. Very few worms found on post-mortem examination.

No. 11 was killed on 27. i. 28, 88 days after dose of Nema. Post-mortem examination showed all internal organs apparently normal. A fairly large number of worms was found.

Result.

A considerable drop in the egg count in most of the sheep dosed.

Table I. *Showing worms found on post-mortem examination of sheep killed after treatment with tetrachlorethylene (Nema).*

Sheep No.	9	8	4	5	0	10	11	Where found
Haemonchus contortus	—	50	—	—	20	Many	8	In abomasum
Ostertagia circumcinta	—	—	—	—	—	Very many	250	,,
Trichostrongylus extenuatus	Very few	25	8	—	10	—	75	,,
T. vetrinus	Very few	—	—	25	Many	—	—	In small gut
T. instablis	200	Few	Few		—	—	—	,,
Cooperia Curticii	Few	Few	Very many	230	Few	—	Many	,,
Nematodirus fillicollis	—	—	Few	—	Many	25	—	,,
Capillaria sp.	—	—	—	—	—	20	—	,,
Bunostomum trigonocephalum	122	—	50	—	—	30	—	,,
Strongyloides papillosus	—	—	—	Very many	—	—	—	,,
Chabertia ovina	26	5	1	—	5	—	4	In colon or caecum
Trichuris ovis	—	6	—	3	8	—	—	,,
Oesophagostomum venulosum	—	—	—	—	—	—	1	,,

Summary.

The results of these experiments are, on the whole, disappointing, since they have failed to reveal a reliable treatment for parasitic gastritis of sheep other than that caused by *H. contortus*, but they do confirm the experiments of other workers in that there are several drugs available which are more or less effective against this worm. Of these perhaps copper sulphate would seem to be in many ways the best for general use, since it is cheap and safe, and can be easily given either dry in capsules or in solution. Better results are obtained when two doses are given, and probably the interval of 7 days between the doses could, with advantage, be shortened to one or two days. The addition of sodium arsenite did not, in these experiments, increase the efficacy of the copper sulphate.

Carbon tetrachloride, given in 12 c.c. doses would seem to be effective against *Haemonchus*. In such doses it is rather expensive, and although in these experiments toxic symptoms were not observed, deaths from smaller doses have been recorded by others.

Tetrachlorethylene (Nema) given in capsules containing 5 c.c. gave, in most cases, good results against *Haemonchus*, and except in Sheep 10 reduced the egg count very considerably. It did, however, cause considerable constipation, and in a few instances post-mortem examination revealed some inflammation of the small intestine. The administration of a laxative with or shortly after the Nema therefore appears to be indicated.

From Table I above it will be seen that *Bunostomum trigonocephalum* were found in three animals after the administration of this

drug, which does not appear, therefore, to be effective for hookworm in sheep.

The results so far obtained with petroleum ether seem promising but further trials are necessary before any definite conclusion can be arrived at.

Turpentine, chloroform and linseed-oil mixture, chloramine T. sodium pentasulphide, arsenic and monsol do not appear to be effective.

A proprietary copper and arsenic mixture, and copper with arsenic and muriatic acid would not appear to have any advantage over copper alone.

Gow's tobacco powder (which is said to be composed of tobacco with 33 per cent. of sulphur) gave promising results, but tobacco alone, sulphur alone, and tobacco and sulphur in the same doses and in the same proportions as in Gow's powder, did not produce any decrease in the egg count.

The poor results obtained from drugs mixed with the food are disappointing, as this method of administration would be extremely useful in the treatment of large flocks of sheep.

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(*Received October 18th*, 1929.)

THE MAINTENANCE REQUIREMENT OF THE FATTENING COCKEREL WITH A NOTE ON A PROPOSED NEW METHOD FOR THE DETERMINATION OF THE SURFACE AREA OF BIRDS.

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The data required for the calculation of the ration of a fattening animal are (1) the maintenance requirement, and (2) the production requirement. The former is usually written Am, where A is the surface area of the animal and m is its maintenance requirement per unit area per unit time. The production requirement may be written gc, where g is the live weight increase per unit time and c the nett energy contained in the food required to make unit weight of gain. Then, if R is the nett energy of the ration,

$$R = Am + gc. \qquad \text{.....(1)}$$

This equation has been made use of by Prof. T. B. Wood(1) for determining the maintenance requirement of sheep, and it was at his suggestion and under the general direction of Mr E. T. Halnan that the following work was undertaken.

The investigation was designed to take advantage of the fact that cockerels can be forcibly fed with a cramming machine, and that under this treatment they will make considerable live-weight increases in a short period. The uncertainty with the production requirement term gc in equation (1) was avoided by finding by analysis the actual gain of calorific value made by each bird as the result of fattening. This was done by killing and analysing a sample group before the investigation began and analysing each bird after the fattening period: the product of the change in live weight and the difference of calorie value per unit weight of carcase after fattening then gave directly the loss or gain of calorific value. If this is done, equation (1) becomes

$$KR = Am \pm c, \qquad \text{.....(2)}$$

where R = weight in gm. of food fed;
K = net energy in calories per gm. of food;
A = surface area in sq. m.;
m = maintenance requirement in calories per sq. m. per hour;
c = calorie equivalent of gain of body substance per hour.

It is obvious that the maintenance requirement under conditions of fattening changes as the fattening proceeds, since the general level of metabolism is raised. For this reason it was decided to conform to commercial conditions as far as possible so that the value obtained would represent the average maintenance requirement over the normal period of commercial fattening.

The surface area of the cockerels was found by using the formula of von Meeh (2), $A = KW^{\frac{2}{3}}$. The value of the constant K was determined directly by measuring the surface area of birds of known weight by a method to be described later. It was found to be $K = 9{\cdot}3$.

REPORT OF INVESTIGATION.

Twenty-four Light Sussex cockerels, bred from the same stock and of the same age, were used. Initially they were in store condition. Following commercial practice they were store fed for 10 days before cramming began, being given 20 gm. daily of a mixture of Sussex ground oats and dried milk in the proportion oats : milk = 13 : 3. This was made into a stiff paste with water. The oats was a good sample, and foreign matter was removed by sifting; the milk was a New Zealand full-cream powder and was readily soluble in water; drinking water was also available during this preliminary period.

After 10 days' trough feeding, 4 birds were selected at random, killed, and analysed. The gut contents were discarded and the whole of the remainder ground through a mill. Samples of this were dried under toluol and alcohol and ground again twice, the final product being a dry, fine powder.

The 20 remaining birds were divided into 4 groups, each group of 5 occupying a wire cage, so as to have slightly more freedom than is customary in commercial practice. A Neeves' cramming machine was used for feeding. The feed consisted of a mixture of Sussex ground oats, dried milk and water in the proportions 13 : 3 : 20 respectively, the result being a fairly fluid paste. It was found impossible to calibrate the cramming machine so as to deliver a known weight of food accurately, but with practice an approximately correct ration could be given. The exact weight was determined by weighing the bird before and after feeding.

Throughout the trial the birds remained in excellent condition, apart from two deaths by accident. After 4 days' cramming the birds were killed, plucked and put into cold store at 0° C. They were taken out at short intervals, sampled and analysed in the manner described for the sample group. No signs of decomposition were apparent in any of the birds when taken from the refrigerator.

Results.

Table I. *Analysis of Food.*

	Sussex ground oats (%)	Whole milk (dried) (%)
Water	11·69	0·75
Protein	12·22	25·26
Fat	5·41	22·45
Carbohydrates (by difference)	58·50	45·87
Ash	3·09	5·67
Fibre	9·06	—

From the data given, an equation of the form of equation (2) may be constructed for each bird:

No. of bird	
110	K 2191 = ·2078 B + 2149
214	K 2126 = ·1949 B + 2136
251	K 1984 = ·2072 B + 1769
162	K 1046 = ·1900 B + 450
115	K 1119 = ·1727 B + 574
106	K 1088 = ·1727 B + 747
225	K 999 = ·1766 B + 860
173	K 1044 = ·1674 B + 893
221	K 756 = ·1503 B + 279
126	K 804 = ·1876 B + 824
114	K 842 = ·1876 B + 535
108	K 810 = ·1870 B + 789
153	K 789 = ·1730 B + 416
158	K 480 = ·1597 B − 356
90	K 440 = ·1627 B − 588
154	K 444 = ·1818 B − 860
183	K 425 = ·1629 B − 287
100	K 427 = ·1660 B − 642

where B = maintenance requirement in calories per sq. m. per 14 days;

K = net calories per gm. of dry feed.

Each of these equations was multiplied in turn by (1) the coefficient of K, and by (2) the coefficient of B (the method of least squares). By addition, two composite equations were obtained from which the values of K and B were extracted.

The values found were:

K = 1·8 calories per gm. of food (Sussex ground oats : milk 13 : 3).

B = 22·1 calories per sq. m. per hour.

This value of B is in fair agreement with that found by Gerhartz (3), who obtained values from 22·0–25·9 per sq. m. per hour for hens.

It will be noted that, except in two cases, the fattening process was accompanied by a loss of protein. These two cases occur in the group

Table II.

No. of bird	Group	Initial empty carcase wt. (gm.)	Final empty carcase wt. (gm.)	Gain in carcase wt. (gm.)	Initial Protein (%)	Initial Fat (%)	Final Protein (%)	Final Fat (%)	Gain of protein (gm.)	Gain of fat (gm.)	Gain in calories: protein × 5·7 fat × 9·5	Area of bird sq. cm.	Wt. of dry food fed (gm.) In 14 days
219	0	2179	2179	—	23·71	3·94	—	—	—	—	—	—	—
12		2086	2086	—	22·56	4·57	—	—	—	—	—	—	—
189		2098	2096	—	19·54	4·12	—	—	—	—	—	—	—
129		2197	2197	—	24·63	3·86	—	—	—	—	—	—	—
110	1	2722	3415	+693									
214		2460	3062	+602			16·93	10·56	− 37·2	+248·5	+2149	2078	2191
251		2725	3285	+560	22·61*	4·12*	17·89	10·8	− 8·4	+229·3	+2130	1949	2126
127		—	—	—			16·72	10·31	− 66·9	+226·3	+1769	2072	1984
112		—	—	—					Died through accident in feeding				
									Died of pneumonia				
162	2	2589	2757	+168									
115		2196	2301	+105			18·36	7·31	− 79·2	+ 94·8	+ 450	1901	1046
106		2182	2357	+175	22·61*	4·12*	17·21	9·18	−100·5	+120·7	+ 574	1727	1119
225		2266	2398	+132			17·08	9·46	− 90·7	+133·0	+ 747	1727	1088
173		2070	2249	+179			17·46	10·01	− 93·6	+146·6	+ 860	1766	999
							17·20	10·14	− 81·2	+142·7	+ 893	1674	1044
221	3	1812	1791	− 21									
126		2614	2525	− 89			23·45	6·15	+ 10·2	+ 35·5	+ 279	1503	756
114		2559	2605	+ 46	22·61*	4·12*	23·00	7·94	− 10·3	+ 92·8	+ 824	1876	804
108		2181	2201	+ 20			18·79	8·26	− 89·0	+109·7	+ 535	1867	842
153		2277	2319	+ 42			18·82	8·22	− 78·9	+ 91·1	+ 416	1870	810
							25·02	7·17	+ 65·4	+ 72·5	+ 316	1730	789
158	4	2056	1925	−131									
90		2039	1854	−185			21·03	4·36	− 60·0	− 0·8	− 350	1597	480
154		2581	2195	−386	22·61*	4·12*	20·92	3·73	− 73·2	− 14·8	− 558	1627	440
183		2162	2008	−154			19·87	4·75	−147·5	− 2·1	− 860	1818	444
100		2276	2081	−195			19·76	5·68	− 92·0	+ 25·0	− 287	1629	425
							19·38	4·46	−111·3	− 0·9	− 642	1660	427

* Average of group 0.

receiving just sufficient food to provide for maintenance, with, as is shown in three cases, a slight surplus for production. From the theoretical standpoint one would expect the loss of protein to occur in the sub-maintenance group owing to the calls of the body on this material for the necessary energy for maintenance. Histological evidence also shows that as fattening proceeds, fat globules appear within the actual muscle fibres themselves, indicating a replacement of muscle substance by fat. The loss of protein shown in the groups receiving the high production rations indicates that this process had occurred in the cases under consideration.

A NOTE ON A PROPOSED NEW METHOD FOR DETERMINING THE SURFACE AREA OF BIRDS.

By E. T. Halnan, M.A. and B. A. Southgate, B.A.

In considering the methods in use for the determination of the surface area of dogs and farm animals it quickly became apparent that such methods were inapplicable to the fowl. Owing to the elasticity of the skin, methods based on measurement of the skin after its removal from the body are bound to be inaccurate, and the varying thickness of the skin in different parts of the body renders attempts to correlate weight of skin with surface futile. Rubner had met with this difficulty in his experiments, and had attempted to overcome it by trimming off the sickle and tail feathers, dipping the body of the bird in paraffin wax, thus causing the feathers to lie flat against the body, and by covering the surface thus produced with squares of paper. A method was therefore sought for on entirely new lines, and after much thought it was decided to seek for a solution of the problem by taking advantage of the absorbability by the skin of chemical substances applied to it. Preliminary trials with various substances were carried out, and eventually potassium permanganate was adopted as being most likely to give reliable results.

Method.

The bird was killed by the stab method in order to avoid the complication caused by the swelling of the neck which takes place if it is killed by dislocation of the neck. After plucking, the skin was brushed over with a mixture of alcohol and ether to remove the surface film of oil. The bird was then hung up by its feet and painted with a strong aqueous solution of potassium permanganate. It was found possible to obtain a uniform distribution, as judged by the colour, if the preliminary washing with ether, and alcohol and ether, had been thoroughly carried

out. The shanks, feet, and head were left unpainted. When dry, rectangles were marked out on the skin with Indian ink in conveniently flat places, as much of the surface as possible being so measured and the areas marked being distributed over different parts of the body. These rectangular pieces of skin were removed with a razor, and finally the remainder, except that covering the head and shanks, was similarly removed. The measured pieces and the remaining skin were first separately dried in the water oven, heated over a small flame in silica evaporating basins (dilute nitric acid being added to assist combustion), and finally reduced to an ash, in which the $KMnO_4$ was retained as manganous oxide. This was estimated by the bismuthate method.

This process, originally described by Schneider (4), has been improved and standardised by Reddrop and Ramage (5) and their modified method was used. The process is based on the fact that a manganous salt is oxidised in the presence of excess nitric acid to permanganic acid by sodium bismuthate. The permanganic acid being stable in nitric acid solution when cold, the excess sodium bismuthate can be filtered off and the potassium permanganate in the filtrate estimated. In practice, the ash was digested several times with 25 per cent. nitric acid to dissolve out the manganous oxide, the insoluble residue was filtered off and the solution heated with 5 gm. of sodium bismuthate. A small quantity of sodium bisulphate was added to clear the solution, which was then boiled for 30 minutes. On cooling to 20° C., excess sodium bismuthate was added and the excess removed by filtration through a Gooch crucible fitted with an asbestos pad, previously ignited and washed. The filtrate was made up to a known volume and fractions of it titrated against 0·1 N $FeSO_4$. By comparing the manganese content of the measured and the unknown areas, the whole of the surface area of the bird could be calculated. The area of the head was determined by direct measurement. The area of the shanks and feet was not included in the whole area of the bird since the heat lost by radiation from them is comparatively small. Determinations carried out on four cockerels gave for the constant in the equation $A = KW^{\frac{2}{3}}$ values of 9·30, 9·20, 9·37 and 9·33, with an average of $K = 9{\cdot}3$.

Rubner (6) gives as the mean of a number of determinations for Wyandotte hens, $K = 10{\cdot}45$, and quoted Regnault as having obtained an identical figure. Kukein (7) gives $K = 10{\cdot}43$. Although not definitely stated, it is probable that these values were obtained by including the area of the shanks in that of the whole bird. The average value of 9·3 found in this investigation is increased to 10·58 when the area of the

shanks and feet (found by direct measurement) is included, and this is in fair agreement with the result of Rubner.

Summary.

(1) The maintenance requirement of Light Sussex cockerels has been found by a feeding trial and comparative slaughter method. The maintenance requirement found was 22·1 calories per sq. m. of surface per hour. The net energy of the ration fed (Sussex ground oats : whole dried milk = 13 : 3) was 1·8 calories per gm.

(2) A new method of determining the surface area of birds is described.

(3) The value of K in the equation area $= K$ weight$^{\frac{2}{3}}$ has been found by this method to be 9·3.

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(*Received November 28th*, 1929.)

MILKING AT THREE EIGHT-HOUR INTERVALS AS A MEANS OF INVESTIGATING VARIATIONS IN THE FAT AND SOLIDS-NOT-FAT.

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(With Two Text-figures.)

In November of 1927, and starting with one cow, we set out to milk twelve cows thrice daily at equal intervals throughout their lactations. The hours of milking were 6 a.m., 2 p.m. and 10 p.m. Samples of the milk of each cow were taken at each milking and tests made for fat and solids-not-fat. The Gerber method was applied for fat tests, using recognised calibrated glass. Veith's lactometer was used for calculating specific gravities and total solids with the aid of Richmond's scale.

Sampling and tests were duplicated at intervals to secure confirmation. The milks for a 2 p.m., 10 p.m. and 6 a.m. milking were tested all together on the day of the 6 a.m. milking. Preservative was not used except on three separate days. A total of 10,689 samples was tested for fat and a total of 10,617 for specific gravity.

Table I gives the list of cows in their order of calving.

Table I.

Name	Date of calving	No. of calves	Weeks in milk
Rockrose 7	Nov. 29th, 1927	2	41
Cherry 8	Dec. 4th, 1927	2	41
Rockrose 8	Dec. 6th, 1927	1	46
Strawberry 5	Dec. 23rd, 1927	1	56
Fillpail 7	Dec. 30th, 1927	1	39
Duchess 7	Jan. 4th, 1928	3	40
Fillpail 6	Jan. 8th, 1928	4	45
Ruby 4	Feb. 5th, 1928	5	39
Duchess 9	Feb. 9th, 1928	3	38
Rockrose 6	Feb. 9th, 1928	2	43
Rockrose 5*	Apr. 11th, 1928	4	44
Rockrose 4	May 3rd, 1928	5	37

* This cow remained in milk for several weeks more than are recorded here, but samples were not taken after the 44th week.

The management of the twelve cows was in general the same as that of the whole herd. During the winter the herd was brought to the cow house about 1.30 p.m. and returned to the field about 8.30 the following morning.

When the ground was snow-covered or badly frostbound the cattle remained in the yard while the cow house was cleaned and were then brought in again. Automatic drinking-bowls operate throughout the cow house. The herd is turned to grass about the end of April and remains out till mid-October. Cake is fed to all cattle giving more than 3 gallons per day in the summer, and the grass is supplemented when necessary with green fodder fed in the cow house.

The herd of the University Farm is a Graded Shorthorn herd of 20 years' standing, and all heifers in the herd in July 1929 were entered in or were eligible for entry in Coates's Herd Book. The maximum number of cows in milk at any one time is twenty, and calving takes place from October to April inclusive. Heifers calve at $2\frac{3}{4}$ to 3 years. Tuberculin testing was introduced at the herd's inception and has been carried out every year to date.

The objects of the work for the purposes of this report were as follows:

1. To collect evidence on the effect of thrice-daily milking on the yield of individual cows in this herd and on the herd as a whole.

2. To collect evidence on the effect of three 8-hour intervals on butter fats.

[Under this heading is discussed the subject of the variation of fat percentages in individual cows and in bulk milk.]

I. The effect of thrice-daily milking on yield.

Various estimates have been made as to the increase in yield of herds due to thrice-daily milking, estimates ranging from 15 to 25 per cent.

Mr Holt Thomas(1), speaking at the Farmers' Club in June 1927, states, "without having definite data on the subject I would put the increase as between 15 and 25 per cent." He goes on to say that if a herd contains enough heavy yielders it must be absolutely commercial to milk three times, implying, presumably, that an increase of 15 to 25 per cent. renders it so.

Mr Boutflour(2), in his remarks at the close of Mr Holt Thomas' address, stated that in a forty-cow dairy under thrice-daily milking about 75 per cent. of the herd showed a definite increase, while 25 per cent. did not increase at all. This statement is based on experience with between 2000 and 3000 cows.

Ragsdale, Turner and Brody(3) state as their experience under purely experimental conditions that cows milked thrice daily would produce 110 per cent. and those milked four times daily 116 per cent. of those milked twice daily. In their work they add that experimental procedure

and stage of lactation caused a decrease in the production of milk to an average of 73 per cent. of that produced at the start.

A comparison between the yields of the nine cows in this trial under thrice-daily milking with the yields of the same cows in previous lactations back to their first has been made and the figures are given in Table II. All yields have been standardised by the use of Sanders'(4) method of standardising milk yields.

The standardised yields of the three heifers in the trial are also shown.

It is seen that the average increased yield of the nine cows in their thrice-daily milking year over the yield in their preceding lactation is 19·3 per cent. The figures comprising the average range from a decrease of 21·1 to an increase of 41·8 per cent.

Duchess 9 shows the largest increase, namely 41·8 per cent. This cow was fed throughout the winter according to Boutflour's principles. She did not, however, receive any appreciable quantity of cake before calving beyond what is customary in the herd, but she did get Glauber salts and ginger both before and after calving. The increase in yield must be attributed in part to thrice-daily milking and to other factors of which the chief may have been the method of feeding.

Duchess 7 shows the second largest increase, namely 40·4 per cent. This cow was also fed and managed throughout the winter entirely according to Boutflour's principles, and including the whole of the steaming-up process. Duchess 7, however, produced a premature calf in early life, with the result on her subsequent yields that can be seen by reference to the table. She made a very slow recovery, and it is possible that her lactation yields were adversely affected by that state of affairs. Accordingly, her increase under thrice-daily milking may, to some little extent, be an artificial one due to her yields in lactations preceding the thrice-daily milking lactation being abnormally low.

The only other result requiring comment is the decrease in the case of Rockrose 5. This cow, in common with most of her family, has been always a very heavy milker and, up to the present year, a regular breeder. After being served in July 1928 she failed to hold to her service and is not now due until November 2nd, 1929. She finally calved about that date. During the thrice-milking trial she never looked well, and although she is a cow that normally carries little flesh she looked poorer than usual in the year in question. The result is apparently reflected in a much reduced standardised yield.

The increases in the case of the other cows do not call for special comment at this stage.

Table II.

Name	Yield with 1st calf: Actual	Yield with 1st calf: Standardised	Yield with 2nd calf: Actual	Yield with 2nd calf: Standardised	Increase or decrease	Yield with 3rd calf: Actual	Yield with 3rd calf: Standardised	Increase or decrease	Yield with 4th calf: Actual	Yield with 4th calf: Standardised	Increase or decrease	Yield with 5th calf: Actual	Yield with 5th calf: Standardised	Increase or decrease	Increase in thrice-milking lactation over preceding lactation (%)
Fillpail 7	6853¼	9,416¼	—	—	—	—	—	—	—	—	—	—	—	—	—
Rockrose 8	6980	8,774¼	—	—	—	—	—	—	—	—	—	—	—	—	—
Strawberry 5	9076½	9,720½	—	—	—	—	—	—	—	—	—	—	—	—	—
Cherry 8	6925⅜	8,788⅜	8,832¾	9,828	+1039⅛ +11·8 %	—	—	—	—	—	—	—	—	—	11·8
Rockrose 6	9593¼	11,281½	12,173	13,110	+1828¼ +16·2 %	—	—	—	—	—	—	—	—	—	16·2
Rockrose 7	6358¾	7,789½	8,970¼	10,190	+2400½ +30·8 %	—	—	—	—	—	—	—	—	—	30·8
Duchess 9	6360¼	8,179¼	6,279¾	7,121½	−1058 −12·9 %	10,000¼	10,100	+2978¾ +41·8 %	—	—	—	—	—	—	41·8
Duchess 7	6777¼	7,197½	2,716	3,408	−3789½ −52·6 %	7,324½	6,884¾	+3476¾ +10·2 %	9,734½	9668¾	+2784 +40·4 %	—	—	—	40·4
Fillpail 6	8627¾	10,068½	7,360	8,000	−2068½ −20·5 %	8,066	7,727¼	−272¾ −3·4 %	10,545¾	9691¼	+1964¼ +25·4 %	—	—	—	25·4
Rockrose 5*	7960	9,201¾	9,658¾	10,006½	+804¾ +8·7 %	11,660¾	12,138¾	+2132¼ +21·3 %	14,857½	9568¼	−2570½ −21·1 %	—	—	—	−21·18
Rockrose 4†	9835½	11,566½	9,793¼	9,470	−2096½ −18·1 %	11,168	10,376	+906 +9·59 %	10,305	9954½	−71 −0·68 %	11,545	11,671	+1716¼ +17·2 %	17·2
Ruby 4	5616¾	7,127½	6,954¾	7,038¼	−89¼ −1·2 %	7,572¼	7,360¼	+322 +4·5 %	7,591¼	7606½	+246¼ +3·3 %	9,345	8,457¼	+8508 +11·2 %	11·2

Average increase of 9 cows 19·9.

* This cow was milked thrice daily for about three months in the summer during her third lactation.
† This cow was milked thrice daily for about three months in the summer during her third and fourth lactations.

An estimate of the increase in yield which may be due to thrice-daily milking in the case of the three heifers in the group has been made by standardising the yields of heifers in the herd with completed lactations back to the milk recording year commencing October 1st, 1923. The average standardised yield of eleven heifers for the period stated was 8730 lb.

Table III shows the increase of the three heifers thrice milked over the eleven twice milked.

Table III.

Name	Actual yield	Standardised yield	Increase	%
Fillpail 7	6853¼	9416¼	686¼	7·2
Strawberry 5	9076¼	9720½	890½	9·1
Rockrose 8	6980½	8774¼	44¼	0·5

The average increase of these three thrice-milked heifers is only 5·6 per cent.—rather a disappointing result in view of Boutflour's opinion (2) that heifers show a greater increase than cows under thrice-daily milking.

It must be pointed out, however, that Strawberry 5 and Rockrose 8 both had peas removed from teats during their lactations. Rockrose 8 was so badly affected that for at least one-third of her lactation she was milking only three-quarters. Strawberry 5, however, was a year older than the average for heifers in the herd because of her unobserved failure to hold to a first service.

One factor associated with thrice-daily milking, and probably contributing to the increase brought about, is the longer period taken to reach the maximum yield and the slower rate at which the yield decreases with advancing lactation. A comparison of the number of weeks taken to reach maximum yield as between twice- and thrice-milked cows and heifers has been made and the result is shown in Table IV.

Table IV. *Average lactation week in which maximum yield is reached.*

Twice milked			Thrice milked		
Cows	Heifers	Cows and heifers	Cows	Heifers	Cows and heifers
6·2	4·6	5·75	7·88	5·66	7·33

The following numbers have been used to arrive at the above averages:

55 twice-milked cows;	9 thrice-milked cows;
22 twice-milked heifers;	3 thrice-milked heifers.

The peas mentioned in the case of the thrice-milked heifers were not removed until after their maximum yield had been passed.

These figures do not show the marked difference in the case of cows

or heifers which one is commonly led to expect. The time taken in the case of the twice-milked cows is surprisingly long. As the total number of cattle used is small, one cannot regard the result as more than an indication. The increase in yield in the case of cows is shown to be within expected limits, but in the case of heifers to be much below expectations.

II. The effect of three 8-hour intervals on fat percentages.

"So far as the percentage of fat is concerned, the intervals between milkings constitute the most important of the known factors associated with variations in the composition of milk." The above is an extract from *Variations in the Composition of Milk*, published by the Ministry of Agriculture and Fisheries (5). The view based on a considerable amount of evidence is one which receives probably universal support. Nevertheless, one may be justified in examining the view itself, as well as the evidence on which it is based, in order to ascertain whether the one is a correct interpretation of the facts, and if so, whether the other is sufficiently complete to warrant such an interpretation.

In May 1905 Lauder (6) commenced an investigation into the yield and composition of the milk of the dairy herd at the Midlothian and Peebles County Asylum at Rosslynlee. The reports on this work for the years 1906–7 and 1907–8 are available to me by the courtesy of Dr Lauder, who very kindly sent me his bound copy of all the reports, as the publication is now out of print.

The cattle in this work were milked twice daily, the milk was weighed at each milking and samples of each milking were taken on one day each week.

In 1905–6 and 1906–7, the intervals between milking were $9\frac{1}{2}$ and $14\frac{1}{2}$ hours, whereas in 1907–8 they were 11 and 13. This alteration in the length of intervals is said to have resulted in an improvement in the fat percentage of the morning milking. The average difference between morning and evening in 1905–6 was 0·65, in 1906–7, 0·49, and in 1907–8, 0·22.

Collins (7) has shown by an examination of data obtained from Chelmsford, Edinburgh, Leeds and Newcastle that when the intervals are

12 and 12 hours, morning fat exceeds evening by 0·18 per cent. on the average of 22 tests;

13 and 11 hours, evening fat exceeds morning by 0·33 per cent. on the average of 192 tests;

14 and 10 hours, evening fat exceeds morning by 0·7 per cent. on the average of 18 tests;

$14\frac{1}{2}$ and $9\frac{1}{2}$ hours, evening fat exceeds morning by 1·09 per cent. on the average of 391 tests.

He goes on to say that if a farmer milked his herd 12 minutes earlier in the morning and 12 minutes later in the evening, the milk would be 0·1 per cent. richer in fat in the morning and correspondingly poorer in the evening.

Cruickshank(8) shows the morning and evening milk of a herd of five cows to be as follows:

5.30 a.m.	3·10	True fat percentage based on weight of milk and fat of each cow at each milking.
12 noon	3·94	
5 p.m.	3·08	

Ragsdale *et al.*(3) state: "The data indicate that the percentage of fat and total solids gradually decreases with the lengthening of the interval between milkings until the time interval exceeds 14 to 16 hours. Thereafter there is a slight increase until the 24th and 26th hours, followed again by a gradual decline until the 36th hour."

The above references have been made because they form a working basis in the light of which the results of this particular piece of work can be examined as far as these results are influenced by the factor of interval between milkings.

As this piece of work advanced it became very clear that in spite of there being three 8-hour intervals between milkings, there were factors causing each of the twelve cows to vary in the manner in which they produced milk, both as to its quantity and quality at successive milkings and at certain milkings on successive days.

Viewing this matter in the light of the advice so frequently given to farmers to reduce the night interval as a means of raising morning fats the question arose: "Has not this advice given to farmers resulted in the assumption being made that by shortening the night interval you reduce to an insignificant number the fat percentages falling below presumptive standard in the case of cows individually?" It is proposed to show that there are no grounds for this assumption. In the work under review a total of 10,689 samples was taken and of this number 1262 or 11·8 per cent. fall to or below 2·95 per cent. The distribution of these low fats was as follows:

2 p.m.	4·0 per cent.
10 p.m.	3·16 ,,
6 a.m.	4·60 ,,

These figures can be compared with others obtained by permission of Mr James Mackintosh from the herd of the National Institute for Research in Dairying. The milk of nine cows under twice-daily milking was being

tested twice daily for butter fat. A count of the low fats was made when the cows still had, on the average, about three months of their lactations in front of them. A total number of 3282 samples was taken and of these 462 or 14·07 per cent. fall to or below 2·95 per cent. In view of the rise in fat which takes place towards the end of a cow's lactation, it is doubtful if there would be any significant difference between the final percentage of low fats produced by the thrice- and the twice-milked cows.

Tocher(9) found, as a result of a study of random samples totalling 676, that the percentage below 3 per cent. was 8·3, but states that theoretically over 12 per cent. of the total have a fat percentage of less than 3.

This would appear to indicate that the long *night* interval under twice-daily milking merely serves to concentrate the low fat percentages of individual cows into the morning milking, but that the shortening of that interval will not necessarily remove them altogether.

Table V. *Showing the number of weeks in each cow's lactation when the highest milk yield and the highest fat percentage occurred.*

Name		2 p.m.	10 p.m.	6 a.m.	Total weeks
Rockrose 7	Milk	10	2	28	40
	Fat	9	18	13	40
Cherry 8	Milk	0	11	29	40
	Fat	1	24	15	40
Rockrose 8	Milk	16	3	21	40
	Fat	1	24	15	40
Strawberry 5	Milk	8	6	26	40
	Fat	2	21	17	40
Fillpail 7	Milk	4	0	35	39
	Fat	10	24	5	39
Duchess 7	Milk	18	3	19	40
	Fat	3	35	2	40
Fillpail 6	Milk	0	3	37	40
	Fat	9	8	23	40
Ruby 4	Milk	21	8	10	39
	Fat	11	27	1	39
Duchess 9	Milk	10	16	12	38
	Fat	6	22	10	38
Rockrose 6	Milk	10	19	11	40
	Fat	0	40	0	40
Rockrose 5	Milk	18	0	22	40
	Fat	36	2	2	40
Rockrose 4	Milk	19	4	14	37
	Fat	17	20	0	37
Totals	Milk	134	75	264	
	Fat	105	265	103	

This table does not imply that the highest milk yield was necessarily coincident with the highest fat percentage.

Table V shows the number of times the highest weekly yield of milk and the highest true fat percentage occurred at each milking in the case of each cow. An examination of the figures from which this table

Table VI.

	Dec. 4th to Jan. 1st	Jan. 1st to Jan. 29th	Jan. 29th to Feb. 26th	Feb. 26th to Mar. 25th	Mar. 25th to Apr. 22nd	Apr. 22nd to May 20th	May 20th to June 17th	June 17th to July 15th	July 15th to Aug. 12th	Aug. 12th to Sept. 9th	Sept. 9th to Oct. 7th	Oct. 7th to Nov. 6th	Nov. 6th to Dec. 4th	Dec. 4th to Jan. 1st	% of all samples for the period	Totals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Jan.	4	5	1	—	—	—	—	—	—	—	—	—	—	21	5·0	31
Feb.	3	5	6	—	—	—	—	—	—	—	3	—	—	—	2·1	17
Mar.	—	2	9	20	—	—	—	—	—	—	—	—	—	—	3·3	31
Apr.	5	7	54	49	47	6	—	—	—	—	—	—	—	—	17·5	168
May	21	19	12	51	52	45	5	—	—	—	—	—	—	—	18·5	205
June	5	28	10	13	55	29	32	7	—	—	—	—	—	—	16·6	179
July	—	4	32	16	8	57	15	27	5	—	—	—	—	—	14·7	164
Aug.	—	—	—	13	11	—	60	22	42	3	—	—	—	—	13·5	151
Sept.	—	—	—	—	9	20	1	73	31	31	—	—	—	—	16·5	165
Oct.	—	—	—	—	—	—	20	—	19	20	25	—	—	—	11·1	84
Nov.	—	—	—	—	—	—	—	15	—	—	—	16	—	—	7·2	31
Dec.	—	—	—	—	—	—	5	12	4	—	—	—	15	—	11·8	36
Totals	38	70	124	162	182	157	138	156	101	54	28	16	15	21	—	1262
% of all samples for the period	16·8	13·4	17·1	19·3	21·1	17·5	13·6	15·4	10·0	5·4	3·2	2·5	4·1	7·9	—	—

is compiled shows that no one particular milking was the means of the greatest quantity of milk being produced uninterruptedly throughout the lactation, but that individual cows gave the greatest quantity at one milking for several weeks, and that then a gradual change took place resulting in a greater yield at another milking for a number of weeks.

Similarly with fat percentages.

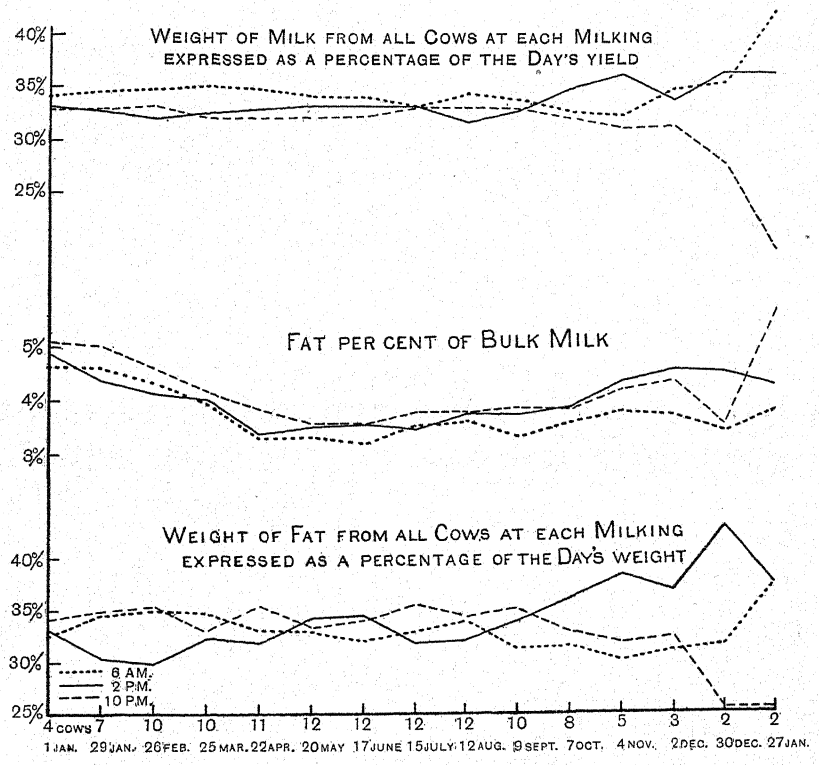


Chart I.

Table VI is a frequency table showing the number of low fats relative to month of year and month of lactation during 14 months of the work expressed as a total and as a percentage. The numbers are those of individual cows, not of bulk milk, so that advancing and closing lactations of the individual cows affect the figures.

Chart I shows the average weight of milk and fat at each milking of all cows per 4-weekly period expressed as a percentage of the average day's yield for that period. The true fat percentage at each milking per 4-weekly period is also shown.

It is shown, then, that in practice the long interval is not itself solely responsible for the number of low fat percentages in the milk of individual cows. It is proposed to pursue this matter farther.

Two of the cows selected for thrice milking were milked, in their following lactation, twice daily at the following hours for 27 weeks of their lactation. During the first 12 weeks at 6 a.m and 10 p.m., *i.e.* with intervals of 16 and 8 hours. During the remaining 15 weeks at 6 a.m. and 8.30 p.m., *i.e.* with intervals of $14\frac{1}{2}$ and $9\frac{1}{2}$ hours. The long interval in this case was from morning to night not from night to morning.

Table VII shows the true fat percentage per week at each milking over a period of four weeks for each cow. The figures refer to the 16th, 17th, 18th and 19th lactating week of one cow and the 17th, 18th, 19th and 20th week of the other.

Table VII.

Week ending	Morning (6 a.m.)	Evening (8.30 p.m.)
Mar. 24th	3·34	3·87
„ 31st	3·38	3·17
Apr. 7th	3·09	3·20
„ 14th	3·13	3·18
Mar. 24th	3·75	3·55
„ 31st	3·82	3·46
Apr. 7th	3·64	3·59
„ 14th	3·51	3·59

When the figures from which this table is compiled are examined it is seen that at 26 milkings out of a total of 112, the fat percentage of the night milking after the longest interval rose above the fat percentage of the morning milking.

Table VIII. *True fat percentage for each week of four.*

Week ending	6 a.m.	10 p.m.	Difference
Dec. 30th	4·42	3·39	1·03
Jan. 6th	4·20	3·27	0·93
„ 13th	3·98	3·07	0·91
„ 20th	4·11	3·00	1·11

Collins' figures (7) show that the difference in morning and evening fat percentage is 1·05 to 1·09 when the intervals are $14\frac{1}{2}$ and $9\frac{1}{2}$ hours with the night interval the longest.

Table VIII shows the true fat percentage of the bulk milk of two cows at each milking for each week of four. The difference between the percentages is also shown.

The true fat percentage for the bulk milk of the whole four weeks is 6 a.m., 4·23; 10 p.m., 3·15; with a difference of 1·08. Similar figures for both cows for the whole 27-week period of the trial are 6 a.m., 3·94;

10 p.m., 3·11; with a difference of 0·83. The figures in the table relate to 16- and 8-hour intervals, those for the whole period relate, as to 12 weeks, to 16- and 8-hour intervals and as to 15 weeks to 14½- and 9½-hour intervals.

Ingle (10) shows that in twice-daily milking where the intervals are 16 and 8 hours, the former being the night interval, the following relationship exists between the morning and evening milk and the morning and evening fat percentage.

	a.m. 16 hours		p.m. 8 hours
Ratio of milk	1·64	to	1.
Ratio of fat content	1	to	1·97.

In the case of the two cows under discussion the ratios are shown in Table IX.

Table IX.

Week ending		p.m.	a.m.		p.m.	a.m.
Dec. 30th	Ratio of milk	1·43	to 1	Ratio of fat	1	to 1·32
Jan. 6th	,,	,, 1·39	to 1	,,	,, 1	to 1·28
,, 13th	,,	,, 1·50	to 1	,,	,, 1	to 1·29
,, 20th	,,	,, 1·44	to 1	,,	,, 1	to 1·35

It is suggested that the above figures which have been compared with Collins' and Ingle's are sufficiently significant to justify a further step being taken in this discussion.

The effect of a long interval has been studied by Bartlett (11) from the point of view of the yield of milk and fat produced after that interval. He suggests as the result of his observations on milk secretion that in the case of high yielding cows and of heifers the rate of secretion between the afternoon and morning milking is greater than the udder capacity, with the result that re-absorption of part of the milk, namely the fat, takes place. He tested this theory by selecting from the herd four high yielding cows, two of which in the cowman's judgment had high udder pressure and two low. He found that the former produced on an average for the first 3 months of their lactations a greater quantity of fat at the evening than at the morning milking, whereas in the case of the low pressure cows the reverse was the case.

The yields of the cows he selected were as follows:

Table X.

	High udder pressure		Low udder pressure	
	Milk (lb.)	Fat (lb.)	Milk (lb.)	Fat (lb.)
a.m.	25·1	0·720	19·6	0·668
p.m.	18·5	0·735	11·7	0·500
	43·6	1·455	31·3	1·168

This question of re-absorption has been investigated in the case of the twelve cows in this trial with a view to a suggestion being made as to the real cause of low fats in morning milk under ordinary twice-daily milking conditions.

In the case of each cow throughout the lactation period, a count has been made of the number of weeks in which the highest milk yield was associated with the highest weight of fat. Of a total of 508 weeks this association of highest milk yield with highest fat yield occurred in 333 weeks or 65·5 per cent. Further, of the total number of weeks, this association occurred at the 6 a.m. milking in only 167 cases, that is, 32·8 per cent.

[A study of the foregoing results together with what is to follow leads the writer to suggest that there is some factor operating at night which tends to a low production of fat in milk. The greater the quantity of milk produced during the night, the lower will be the percentage of fat in the morning milk—because fat is not produced at the same rate as at other parts of the day.]

Table XI. *Yield of milk and fat at each milking for first* 12 *weeks of lactation of Rockrose* 5 *and* 4.

	2 p.m.		10 p.m.		6 a.m.	
	Milk (lb.)	Fat (lb.)	Milk (lb.)	Fat (lb.)	Milk (lb.)	Fat (lb.)
Rockrose 5	125	5·418	111½	4·592	125¼	4·083
	137¾	6·235	136½	5·637	157¼	5·409
	145	5·773	157½	5·627	162¼	4·722
	160	7·147	153¼	4·887	159¼	4·784
	166⅔	7·416	153⅓	4·915	176	5·311
	167¼	7·342	152½	4·283	177½	4·508
	172¼	7·727	155⅔	5·197	168⅓	4·427
	166½	7·099	156¼	5·559	170¾	4·763
	146½	5·638	140¾	4·139	161¼	5·228
	144¾	5·293	141¼	4·998	149	5·236
	150¾	6·462	131¼	5·132	137¾	3·754
	143½	4·685	137¼	6·132	126¾	3·084
Rockrose 4	133¼	5·343	118	4·648	115¼	3·540
	160½	5·781	148½	5·285	158¾	4·812
	169	6·135	167	6·275	166¼	5·000
	182	6·433	175¼	6·687	164	4·792
	167¾	6·011	168¼	6·129	162¼	4·879
	159	5·713	153¾	5·580	151¼	4·368
	153¼	4·967	171⅓	7·066	152¾	5·332
	156	5·121	158¾	6·432	137¼	4·149
	151¼	4·837	162	6·695	138¾	4·474
	136½	4·540	141¼	5·367	143¾	5·466
	129¾	4·798	133¼	4·355	134½	4·141
	131	4·987	118½	4·124	121¾	3·429
	3655	140·901	3542½	129·741	3618	109·691
	21·88	0·843	21·21	0·777	21·65	0·657
		3·85 %		3·67 %		3·03 %

The yields of the two heaviest milking cows in our group of twelve have been examined during the first 12 weeks of their lactations. The average daily yield of the two together during this period was 64·74 lb. Table XI shows the weekly yields of milk and fat at each milking of these cows and the average yield of milk and fat at each milking of the two cows taken together.

Table XII. *Yields of fat and milk at each milking for the first* 12 *weeks of lactation of the three heifers—Strawberry* 5, *Rockrose* 8 *and Fillpail* 7.

	2 p.m.		10 p.m.		6 a.m.	
	Milk (lb.)	Fat (lb.)	Milk (lb.)	Fat (lb.)	Milk (lb.)	Fat (lb.)
Strawberry 5	46	2·647	37½	2·245	43½	2·438
	59½	3·363	63	3·637	64½	3·570
	65½	3·065	66¼	3·375	66	3·245
	65	2·378	73¼	3·783	60¾	2·701
	61¾	2·367	68¾	3·515	69¾	3·472
	62½	2·366	68¾	3·133	71¼	3·224
	69	2·629	69	3·091	66¾	2·891
	67	2·466	68	3·078	65	2·513
	64¼	2·416	61½	2·713	65¼	2·602
	69	2·174	68¼	2·786	68¾	2·445
	61¼	1·941	56	1·974	70½	2·509
	57	1·853	56¼	1·979	78	2·592
Rockrose 8	5¾	0·149	6	0·150	9½	0·361
	60¼	3·408	51½	2·845	56	3·013
	60½	3·304	60¼	3·384	61¼	3·403
	63¾	3·350	65	3·517	63	3·037
	59¾	3·084	64¾	3·604	61¼	3·198
	59½	2·752	57½	3·234	57½	2·910
	54½	2·469	51¾	2·672	54½	2·563
	45	2·333	38¼	1·911	43	1·989
	45	2·108	45½	2·353	51	2·408
	47¼	2·211	45	2·152	50¼	2·292
	48¾	2·187	44½	1·951	48	2·091
	47¼	2·173	44	2·149	49½	2·249
Fillpail 7	36¼	1·714	41¾	2·137	48¾	2·352
	69	3·084	68¼	3·767	73½	3·674
	70½	3·242	70¼	3·823	73¾	3·767
	71½	3·567	67¼	3·627	70¾	3·201
	67¼	3·001	66¾	3·325	72	2·775
	62	2·647	66½	3·455	70½	3·063
	62½	2·670	60½	2·850	68¾	2·820
	59¾	2·577	55	2·718	67¾	2·957
	61¾	2·441	59¼	2·804	71¼	2·823
	62¼	2·594	58¼	2·487	68¼	2·521
	62	2·523	57½	2·360	71½	2·432
	58½	2·349	64¼	2·908	71½	2·794
	2088¼	91·602	2066	101·492	2223	98·894
	8·63	0·379	8·55	0·419	9·18	0·408
		4·39 %		4·89 %		4·45 %

It is seen that, although there is not any considerable difference between the average milk yield at each milking, there is a very significant

difference between the fat yields and that the highest fat yield is associated with the highest milk yield. This association occurs at the 2 p.m. milking. In only 5 weeks in 24 are highest milk yields and lowest fat yields associated—and on each occasion this occurred at the morning milking.

The maximum yield per milking of the cows quoted by Bartlett is 25·1 lb., containing 0·720 lb. of fat. The maximum yield of the two heaviest milking cows in our group of twelve was 21·88 lb. containing the maximum quantity of fat, namely 0·843 lb. It appears unlikely that the point at which re-absorption takes place is somewhere between the two maximum yields quoted.

Table XII shows corresponding figures for the three heifers in our group of twelve. The yields at each milking are small, and it cannot be said that the average yields of milk and fat confirm the suggested possibility of the association of high milk yields and high fat yields.

An examination of the table, however, shows that in 21 weeks out of 36 this association is found and that only in 5 weeks is the highest milk yield associated with the lowest fat yield.

Turning to the two cows which were milked twice daily with the long interval from morning to night, we again find the association of highest milk yield with highest fat yield. These two cows cannot be called heavy milkers, as their combined average daily yield during the first 12 weeks of their lactations was only 32·75 lb.

Table XIII shows the average milk yield and fat yield of these two cows together at each milking throughout their lactation and during the first 12 weeks of their lactations. True fat percentages are also shown.

Table XIII.

	a.m.			p.m.		
	Milk (lb.)	Fat (lb.)	Fat (%)	Milk (lb.)	Fat (lb.)	Fat (%)
Average yield throughout lactation	11·57	0·457	3·95	16·27	0·527	3·14
Average yield in first 12 weeks ...	13·45	0·580	4·31	19·30	0·645	3·34

The ratio of milk to milk and fat percentage to fat percentage in the case of the 12-week period is as follows:

	a.m.	p.m.
Ratio of milk	1	to 1·43.
Ratio of fat content ...	1·29	to 1.

The intervals between the milkings were in this case 16 and 8 hours, yet the proportions bear little relation to those put forward by Ingle under the same condition as to length of interval. In other words, in the

case of the two cows in this trial there is a much bigger proportion of fat at the p.m. milking—after the longer interval—than Ingle obtained at the a.m. milking, also after the longer interval. This gives support to the suggestion put forward above that some factor is operating at night tending to a low secretion of fat and, as is indicated by comparing the above proportions with Ingle's, a larger secretion of milk. Hence low fat percentages in the morning milk under twice-daily milking conditions.

It is desired to emphasise one or two points before closing this matter.

It has been shown that there is little difference, if any, between the total number of low fats produced by individual cows under this thrice-daily milking trial and the total number produced by nine other cattle under twice-daily milking. Experience shows that bulk morning milk, under twice-daily milking, frequently falls below the presumptive standard. Reference to Chart II shows that the bulk milk in the case of this herd of twelve only fell below standard about four times during the whole 14 months. It might be assumed from this that it is sufficient to advise the farmer to reduce his night interval, in order to eliminate low fats in his morning milk. It seems very doubtful if the comparatively small reduction in the length of the night interval which can be made in practice is likely to be effective. Bearing this in mind, it would appear to put the farmer in no better position from the point of view of his bulk morning milk, particularly if we can accept the view, after further investigation, that factors are operating at night which tend to a low secretion of fat.

The bulk of the work done to investigate the causes and extent of the variation of the fat of milk has been done on the milk of cows milked twice daily at the intervals commonly adopted in farm practice. Comparatively little has been done on cows milked at 12-hour intervals and less on those milked at three 8- or four 6-hour intervals.

It is on this account that it can be seriously suggested that the view expressed by the Ministry of Agriculture's Publication(5) concerning the effect of interval on fat percentage is based on insufficient evidence, and tends to be misleading as far as the research worker is concerned. There is only one way of studying the effect of a long interval and that is by studying the effect of the different factors operating at successive and equal intervals. This can only be done by milking at two, three or four equal intervals per day.

A further experiment is required, namely, to study the effect of these factors at equal successive intervals on the individual cow in the first place. Only in this way can the effect of these factors be classified as far

as they may concern cows of different ages or cows in the different stages of lactation. Tocher(12) writes as follows: "The only remedies I can see to reduce the variations in the constituents of milk and give as nearly as possible a milk of constant good quality are...(2) To insist upon the selection of good cows—this can only be done by selling cows upon the basis of the yield of butter fat during an entire lactation period for THE PARTICULAR AGE OF COW."

Table XIV.

									Week ending
Duchess 7	2 p.m.	2·90	3·80	3·05	2·95	3·25	2·75	3·25	May 27th
	10 p.m.	3·35	3·35	3·00	3·45	3·10	3·45	3·30	
	6 a.m.	2·95	2·85	3·35	2·85	3·10	2·95	3·15	
Rockrose 7	2 p.m.	3·40	3·40	3·60	3·40	3·10	3·45	3·65	May 27th
	10 p.m.	3·00	3·20	3·85	3·20	3·00	3·45	3·70	
	6 a.m.	3·40	3·35	3·20	3·40	3·50	3·45	3·10	
Cherry 8	2 p.m.	4·00	2·70	3·40	3·10	3·40	2·80	2·00	July 1st
	10 p.m.	2·85	3·35	3·50	2·95	2·85	3·05	2·60	
	6 a.m.	2·60	3·70	4·30	4·30	4·70	3·95	4·80	
Strawberry 5	2 p.m.	3·50	3·00	3·35	3·55	3·35	3·60	2·90	July 1st
	10 p.m.	3·20	3·00	3·60	3·15	2·80	3·00	3·60	
	6 a.m.	3·70	4·35	4·10	4·20	4·45	3·95	4·05	
Ruby 4	2 p.m.	3·15	3·65	3·15	3·25	3·10	3·05	3·40	Sept. 30th
	10 p.m.	4·05	3·95	3·95	4·45	4·20	4·15	4·50	
	6 a.m.	2·65	3·00	2·80	3·15	2·80	3·30	2·90	
Rockrose 6	2 p.m.	3·25	2·70	3·30	2·80	3·45	3·30	3·90	July 29th
	10 p.m.	3·85	3·55	3·40	3·65	3·50	3·75	3·95	
	6 a.m.	2·80	3·35	2·75	2·80	2·00	2·70	2·95	
Rockrose 5	2 p.m.	5·50	5·40	4·75	4·80	6·80	3·75	6·10	Dec. 9th
	10 p.m.	3·45	3·45	4·55	3·80	4·50	2·80	3·25	
	6 a.m.	4·00	2·70	3·05	3·30	2·45	3·60	2·85	
Duchess 9	2 p.m.	3·00	3·90	3·60	2·90	2·75	2·30	3·60	Sept. 30th
	10 p.m.	2·50	4·00	3·60	2·20	3·15	2·65	2·90	
	6 a.m.	4·15	4·20	6·55	4·95	3·90	5·85	4·85	
Rockrose 4	2 p.m.	4·45	4·15	4·35	4·25	4·25	3·95	3·80	Dec. 23rd
	10 p.m.	4·30	3·30	4·00	4·20	4·05	3·85	3·40	
	6 a.m.	3·05	3·40	2·70	2·90	2·80	4·10	3·45	
Fillpail 6	2 p.m.	3·80	2·90	3·20	3·50	3·70	3·10	4·10	May 13th
	10 p.m.	2·75	3·20	3·70	3·10	2·75	3·60	2·80	
	6 a.m.	2·45	3·70	3·40	3·10	2·95	3·10	3·15	

Lack of space forbids a detailed study of the extent of the variation of the fat of milk of individual cows and of the fat of bulk milk. Sufficient can be written, however, to indicate that marked variation in the fat is by no means confined to the cow milked twice daily. Indeed, it is probably not too much to say that individual cows milked at three equal intervals will vary as much as individual cows milked at two unequal intervals—that is, the range of fats over the three milkings is as great as the range over two milkings. A study of the fat percentages of our group of twelve cows shows, however, that at one milking in the three, each individual showed greater consistency and a better percentage

consistently at that one milking from day to day than she did at the other two milkings. It is undoubtedly this factor which has resulted in the bulk milk of these cows maintaining a yield with marked consistency above the presumptive standard at each milking. The amount of variation, however, above that standard is considerable and sufficient to necessitate very great care before advising that the standard be raised.

Table XIV shows the fat percentage of the milk of ten cows at each milking for a week. It will be noticed that the weeks specified range from May to December. Table I shows the calving dates of these cows.

Table XV shows the fat percentage of the bulk milk of twelve cows at each milking for three separate weeks.

Table XV.

								Week ending
2 p.m.	3·60	3·40	3·00	3·34	3·66	3·63	3·21	July 6th
10 p.m.	3·45	3·53	4·14	3·91	3·84	3·98	3·87	
6 a.m.	3·48	3·51	3·47	3·41	3·44	3·56	3·66	
2 p.m.	3·99	2·84	3·38	3·32	3·62	3·67	3·78	July 15th
10 p.m.	3·57	4·13	3·88	3·92	3·81	3·71	3·71	
6 a.m.	3·46	3·79	3·82	4·00	3·82	3·98	3·88	
2 p.m.	3·67	3·72	3·48	3·35	3·41	3·33	3·70	May 27th
10 p.m.	3·25	3·22	3·64	3·44	3·49	3·62	3·32	
6 a.m.	3·31	3·12	3·00	3·27	3·50	3·13	2·98	

Chart II, moreover, shows the extent of the variation of the bulk milk. The fat percentages of the bulk milk are calculated from the percentages of individual cows and are not arrived at by test. They are based on weight of milk and weight of fat of individual cows.

Summary and conclusions.

It has been shown that in this trial the increase in milk yield of the nine cows considered as a herd is within the limits normally expected in the case of thrice-daily milking.

The increase in the case of the heifers is below expectation, and the probable reasons for this are indicated.

The effect of three 8-hour intervals on butter-fat percentage has been studied, and it is shown that as far as the individual cows are concerned there is little difference between the number of low fats produced under thrice milking at equal intervals and twice milking at unequal intervals in the case of the herds specified. It is shown that the reason for the bulk milk of the thrice-milked cattle maintaining a high standard of fat percentage at each milking is that each individual cow maintains a fairly high level consistently at one specific milking, and that this is not the same milking for each cow.

Chart II. Showing daily fat per cent. at each milking of the bulk milk of twelve cows.

It is emphasised that the research worker, who is looking for and trying to distinguish between cause and effect, must not accept too readily the dictum that the long interval under twice-daily milking is the dominating factor as affecting the fat percentage of milk.

Evidence has been brought forward to show that there is good reason for suggesting that there is some factor operating at night resulting in a low production of fat and that this factor will operate whether the NIGHT interval be long or short. When, however, it is long, milk secretion is encouraged at a greater rate than fat secretion, with the result that morning milk is low in fat. That this is due as much to the influence of night as to interval is shown when the position of the long interval is changed. If this can be accepted, then it must no longer be assumed that because a farmer will not, or is not able to alter his hours of milking, he is not doing everything possible to produce milk within the limits of the presumptive or legal standard.

The extent of variation in fat percentage has been briefly indicated, and it is pointed out that marked variation is by no means confined to the cow milked twice daily. Further, it is shown that although the bulk milk of the herd of twelve maintains a level above presumptive standard, the variation above that standard is very considerable.

I wish to express my thanks to Prof. Pennington for his help while the actual trial was in progress and to him and Mr James Mackintosh for their help in the construction of this portion of the report.

Captain Golding and Dr Thompson also afforded me very material help.

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(*Received January 20th*, 1930.)

REPRODUCTIVE DISTURBANCES CAUSED BY FEEDING PROTEIN-DEFICIENT AND CALCIUM-DEFICIENT RATIONS TO BREEDING PIGS.

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(With Eight Text-figures.)

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Introduction.

The investigation here reported was originally commenced in the autumn of 1921 by the late K. J. J. Mackenzie, then Reader in Agriculture at the School of Agriculture, Cambridge. In 1923 the writer took over the management of the investigation as assistant to Mr Mackenzie until the latter's death in 1924. Owing to an outbreak of disease in March of that year, all the experimental animals had to be slaughtered and an analysis of the results so far obtained was made. The results did not appear to be sufficiently conclusive, and it was decided to hold over their publication until the investigation had been continued on the basis of a wider

range between the control and experimental groups, which experience had indicated to be necessary. There was not such a closely controlled technique in the first experiment, and while the results are more easily interpreted in the light of the conclusions to be drawn from the more complete investigation now reported, they are adequately dealt with in a note at the end of the present paper. As a result of the experience gained a new scheme on which the present investigation was based was drawn up and commenced in August 1924. That part of the work which concerned the writer, namely the recording of data concerning breeding performance, growth and general health was carried on till January 1928, when he left the Animal Nutrition Research Institute at Cambridge to join the staff of the Rowett Research Institute, but a few of the remaining experimental animals were maintained until July 1928 to allow a colleague, Dr R. E. Evans, to complete some metabolic observations of pregnant sows on the deficient diets, the results of which have been communicated separately (9).

From 1924 till 1928 the writer worked under the direction of Dr F. H. A. Marshall, to whom he wishes to make acknowledgement not only for general guidance and support, but also for assistance in personally determining the numbers and condition of the ovarian and uterine contents of all sows slaughtered.

Statement of problem.

In most farm animals fertility is very closely connected with the number of ova which are shed at each heat period. In the case of the pig, however, sufficient eggs are normally shed to allow the production of an adequate number of embryos and one of the factors leading to small litters at birth is the occurrence of foetal atrophy during pregnancy. It is a matter of common observation that a considerable number of embryo pigs undergo atrophy at all stages of their development, and in certain cases to such an extent as to interfere with the number of viable piglings born. Apart from the directly practical aspect of its effect upon fertility, there are several aspects of this foetal degeneration which are of considerable scientific interest, and the investigation to be described was undertaken to examine one of its possible causes.

In examining the problem, Hammond (1) had determined that atrophy was not due to bacterial infection, since it commonly occurs in sows in which the uterus is entirely aseptic. He had also demonstrated that it was not due to overcrowding in the uterus. At the inception of the investigation there appeared to be some evidence that nutritional

deficiencies would be found to be at the root of the disturbance, and as protein and calcium are two of the most important constituents required by the developing embryo, it was decided to investigate the effect of their deficiency in the rations of breeding sows.

Since the work was commenced, however, other possible causes have been suggested, of which the most important are the existence of lethal or sub-lethal factors and the absence of the reproductive vitamin E. These are dealt with later under "Discussion of results."

PLAN OF EXPERIMENT.

The plan adopted to study the effect of a deficiency of protein and of calcium was as follows. Three groups of six gilts, all equal as regards breeding and previous feeding and management, which had been controlled by the investigator, were put up for experiment at the age of about 6 months. From that date each group was fed on a different ration, and as they were invariably housed and exercised on concrete they had no access to grass, soil or any other material except the wheat straw with which they were littered. Table I indicates that the control pen was fed a ration which supplied adequate amounts of protein, energy and the more important mineral elements. It also contained the four vitamins A, B, C and D, vitamin E not having been recognised at that time. The calcium-deficient pen was fed exactly the same ration as the control, with the exception of ground limestone. In the case of the protein-deficient pen it will be recognised that in withdrawing the dried blood a certain amount of mineral matter would also be removed, and this was made good by adding a mineral mixture containing approximately the same amounts of the important mineral elements as were present in the blood-meal ash. Commercial cattle feeding cod-liver oil was used as the source of vitamins A and D. Vitamin C was supplied in the form of fresh oranges, which after having the skins removed were put through a mincing machine. Vitamin B was assumed to be present in the cereal grains and pulse of the basal ration.

The rations used were made up on the following principles. A basal ration consisting mainly of barley and maize was provided in order to supply energy without much protein. A very small amount of bean meal was added to improve the quality of available protein without materially increasing its quantity. Wheat offals were omitted on account of their high nitrogen content. To produce the "complete" ration, blood meal was added to the basal ration to meet the protein requirements of an average sized pig gaining 1 lb. live weight per day. Calcium carbonate in

the form of ground limestone was then added to bring the CaO : P_2O_5 ratio to approximately the same as is found in sows' milk. Finally salt was included equal to one-third of the weight of added calcium carbonate.

Table I. *Rations.*

	Control (parts)	Protein-deficient (parts)	Calcium-deficient (parts)	All groups (per head per day)
Barley meal	188	188	188	Cod-liver oil: ¼ oz.
Maize meal	188	188	188	
Bean meal	16	16	16	Fresh orange pulp:
Blood meal	28	—	28	Under 40 lb. 20 c.c.
Ground limestone	7	7	—	40 to 200 lb. 100 c.c.
Salt	2	2	2	Sows 150 c.c.
Mineral mixture	—	1·0555	—	

Analysis of blood meal (%)		Composition of mineral mixture (parts)	
K_2O	0·4820	K_3PO_4	0·733
Na_2O	1·1362	Na_3PO_4	0·409
CaO	0·3424	NaCl	1·430
P_2O_5	0·4223	$NaH.CO_3$	0·416
Cl	0·8631	$CaCO_3$	0·575
Mg.O	0·0390	$MgCO_3$	0·081
Fe_2O_3	0·0858	Fe_2O_3	0·1257
			3·7697 = 100 parts blood meal

For the protein-deficient group the blood meal was removed from the ration and a mineral mixture containing the equivalent of the mineral elements in the blood meal took its place.

The calcium-deficient group received the same rations as the control with the exception of calcium carbonate.

The requirements for protein, energy and calcium were calculated for pigs, making an average live weight gain of 1 lb. per day from weaning to 350 lb., and a table was drawn up showing the live weights at which successive increments of ¼ lb. of the ration were to be made. As there did not appear to be any figures showing the protein and calcium requirements for suckling sows these were not rationed, but were allowed as much food as they would take up to 14 lb. per day. Gilts over 350 lb. before farrowing were allowed 6·75 lb. per day, and sows between weaning and farrowing were allowed 7 lb. per day. Table II shows the requirements estimated for different live weights, the total amount of food fed at these weights and the amounts of protein, energy and calcium supplied in the three rations. It will be seen that in the control group the protein requirement was taken as the standard, and as the nutritive ratio of the requirements varies with increasing weight and as only one ration was

used in each group for all ages, the supply of starch equivalent is somewhat in excess at the heavier weights.

It was soon found that though blood meal produced diarrhoea in young pigs it had a distinctly constipating effect on older animals, and for this reason one scalded bran mash was fed regularly every week to each animal on experiment.

Table II. *Requirements for protein, energy and calcium, of pigs gaining* 1 *lb. live weight per day, with the amounts of these constituents supplied in the experimental rations.*

Live weight (lb.)		50	100	150	200	250	300	350 and over
Meal fed per head per day (lb.)		3·0	4·5	5·25	5·5	6·0	6·25	6·75
Digestible crude protein	Requirement	0·34	0·51	0·60	0·63	0·67	0·70	0·71
	Control	0·34	0·52	0·60	0·63	0·69	0·72	0·77
	Protein-deficient	0·22	0·32	0·38	0·40	0·43	0·45	0·49
	Calcium-deficient	0·35	0·53	0·61	0·64	0·70	0·73	0·79
Starch equivalent	Requirement	2·05	2·89	3·37	3·73	4·19	4·25	5·3
	Control	2·20	3·30	3·86	4·04	4·41	4·59	4·96
	Protein-deficient	2·22	3·34	3·89	4·08	4·45	4·64	5·01
	Calcium-deficient	2·24	3·36	3·91	4·10	4·47	4·66	5·04
Calcium (as CaO)	Requirement	0·02	0·02	0·02	0·02	0·02	0·02	0·02
	Control	0·024	0·036	0·042	0·044	0·048	0·050	0·054
	Protein-deficient	0·024	0·036	0·042	0·044	0·048	0·050	0·054
	Calcium-deficient	0·002	0·002	0·003	0·003	0·003	0·003	0·003

The requirements as above have been calculated as follows: (1) Digestible crude protein from the curves on p. 163 of Wood, *Animal Nutrition* (University Tutorial Press). (2) Starch equivalent from the curves on p. 163 of Wood, *op. cit.* (3) Calcium (as CaO), Orr, "The Importance of Mineral Matter in Nutrition," *Trans. Highl. and Agr. Soc.* (1923), Fifth Series, **35**.

Having established these three ration groups the following breeding arrangements were then organised. Each of the original gilts on reaching sexual maturity was served by the same boar, and out of the six in each group four were slaughtered when 3 months in pig (gestation lasts 4 months). The number of ova shed in each case was determined from the number of corpora lutea, and the number of normal and atrophic foetuses was also obtained. The remaining two gilts were allowed to farrow, and complete records of numbers, weights, etc., were kept. From these young pigs a further group of six gilts was selected which were weaned, reared and served, while on the same ration as their dams and again only two of these were allowed to farrow. When the plan was fully developed there should always have been four sows, six store gilts and six gilt piglings in each group.

All the piglings farrowed were removed from the sow at birth and, in addition to being weighed before they had an opportunity of getting any

milk, they were earmarked and records taken of sex and vigour. Records were also made of the state of the sow's udder and of her general condition and temper. The writer was personally responsible for recording all breeding data, and for the preparation of the experimental rations except when absent officially or on holiday and at such times his place was taken by a reliable worker.

Results.

Tables IV, V and VI give detailed results for the three groups and Table III is a summary of the averages from these groups. Under the heading "killed" are given three columns showing (1) the number of ova shed, (2) the number of normally developed foetuses, and (3) the number of atrophic foetuses, when the sow was slaughtered at the end of the 12th week of pregnancy. Under the heading "farrowed" are given first of all the numbers of foetuses which, at birth, were (1) alive, (2) dead but normally developed and (3) atrophic. The subsequent columns under "farrowed" give the average weight per head of the piglings at birth, weaning and at 12 and 16 weeks old.

In the case of some individual sows records are available both from farrowing and from slaughter. In a few cases some pigs were slaughtered during the first pregnancy and so no farrowing results were obtained. In other cases only farrowing results were available from sows which were still alive at the end of the experiment.

The lines showing average results for each generation or for each group give the weighted averages of all animals involved. In order to obtain a basis for comparison of the proportions of atrophic foetuses in different generations or groups the average number of normal foetuses at slaughter has been reduced or increased to equal 10 and the other figures altered in proportion. In the case of pigs farrowed the number born alive has been taken as the standard and made equal to 10. This percentage method, however, is not applicable to the weights at birth and later.

(*a*) *Control.*

1. In considering these results, it has first to be noted that the factors of major importance in the experiment were successfully controlled. The group on the "complete" ration was taken as control, and the two groups on deficient diets were those under experimental observation. In the attempt to bring the experiment as nearly as possible within the scope of laboratory conditions, it was decided that all pigs should be kept con-

Table III. *Summary of average results.*

Generation		Killed			Farrowed							
					At birth				At weaning (7 weeks)		At 12 weeks	At 16 weeks
					Number			Av. wt.				
		Ova shed	Normal	Atrophic	Alive	Dead	Atrophic	per head (lb.)	No.	Av. wt. (lb.)	Av. wt. (lb.)	Av. wt. (lb.)
Calcium-deficient group												
FIRST	Average	17·83	10·66	1·83	8·4	0·4	0·0	2·41	5·4	22·06	34·45	48·65
	Normal or alive = 10	—	10·0	1·72	10·0	0·48	0·0	—	—	—	—	—
SECOND First litters	Average	15·00	12·0	0·0	11·07	1·0	0·0	2·26	7·0	15·64	24·46	36·47
	Normal or alive = 10	—	10·0	0·0	10·0	0·86	0·0	—	—	—	—	—
SECOND Second litters	Average	14·75	12·50	0·5	9·2	2·6	0·2	2·52	5·0	13·57	21·30	33·38
	Normal or alive = 10	—	10·0	0·4	10·0	2·83	0·22	—	—	—	—	—
SECOND Third litters	Average	—	—	—	4·5	2·25	2·25	2·73	6·0	9·62	11·27	13·40
	Normal or alive = 10	—	—	—	10·0	5·0	5·0	—	—	—	—	—
	Group Average ...	—	11·45	1·18	8·3	1·32	0·47	2·44	5·7	19·02	—	—
	Normal or alive = 10	—	10·0	1·03	10·0	1·59	0·57	—	—	—	—	—
Protein-deficient group												
FIRST	Average	14·30	10·8	0·2	10·1	0·0	0·0	2·47	6·7	18·90	26·47	32·01
	Normal or alive = 10	—	10·0	0·19	10·0	0·0	0·0	—	—	—	—	—
SECOND First litters	Average	12·00	11·0	1·0	10·0	1·3	0·3	2·15	5·7	13·09	10·63	10·0
	Normal or alive = 10	—	10·0	0·91	10·0	1·3	0·3	—	—	—	—	—
SECOND Second litters	Average	12·00	12·0	0·0	8·0	0·5	0·0	2·66	6·8	18·22	25·83	32·74
	Normal or alive = 10	—	10·0	0·0	10·0	0·6	0·0	—	—	—	—	—
	Group average ...	—	11·0	0·29	9·6	0·35	0·06	2·46	6·53	17·71	—	—
	Normal or alive = 10	—	10·0	0·26	10·0	0·37	0·06	—	—	—	—	—
Control group												
FIRST	Average	17·00	10·2	0·4	11·7	0·1	0·0	2·31	7·4	19·52	33·54	55·95
	Normal or alive = 10	—	10·0	0·39	10·0	0·09	0·0	—	—	—	—	—
SECOND First litters	Average	13·00	10·5	0·5	6·33	0·33	0·0	2·66	5·6	22·93	27·75	40·67
	Normal or alive = 10	—	10·0	0·48	10·0	0·53	0·0	—	—	—	—	—
SECOND Second litters	Average	16·83	9·33	1·83	9·0	0·0	0·0	2·78	7·7	17·44	20·45	—
	Normal or alive = 10	—	10·0	1·97	10·0	0·0	0·0	—	—	—	—	—
SECOND Third litters	Average	11·50	8·0	1·0	10·0	0·5	0·0	2·79	7·5	27·26	27·33	33·20
	Normal or alive = 10	—	10·0	1·25	10·0	0·5	0·0	—	—	—	—	—
THIRD First litter	Average	—	—	—	4·0	0·0	1·0	2·82	4·0	22·35	22·12	33·95
	Normal or alive = 10	—	—	—	10·0	0·0	2·50	—	—	—	—	—
	Group average ...	—	9·67	1·07	9·36	0·18	0·05	2·53	6·45	20·91	—	—
	Normal or alive = 10	—	10·0	1·11	10·0	0·19	0·05	—	—	—	—	—

Table IV. *Details of control group.*

Generation	Sow's number	Month killed or farrowed	Killed			Farrowed									
						At birth				At weaning (7 weeks)		At 12 weeks		At 16 weeks	
						Number			Av. wt. per head (lb.)						
			Ova shed	Normal	Atrophic	Alive	Dead	Atrophic		No.	Av. wt. (lb.)	No.	Av. wt. (lb.)	No.	Av. wt. (lb.)
First	3. K. 1	Jan. 25	—	—	—	11	0	0	1·88	3	18·07	2	33·50	1	43·0
	,,	July 25	—	—	—	14	0	0	2·48	11	16·16	2	26·25	2	58·0
	,,	Jan. 26	—	—	—	14	0	0	2·31	11	13·36	2	18·50	2	23·5
	,,	May 26	17	12	0	—	—	—	—	—	—	—	—	—	—
	6. K. 2	Jan. 25	—	—	—	11	0	0	2·54	8	22·14	2	37·75	2	57·5
	,,	July 25	—	—	—	15	0	0	2·66	12	22·23	2	42·75	2	66·0
	9. L. 3	July 25	—	—	—	12	0	0	2·08	2	24·25	1	38·70	1	72·0
	,,	Jan. 26	—	—	—	14	0	0	2·09	7	14·83	4	24·00	2	51·5
	,,	June 26	18	10	1	—	—	—	—	—	—	—	—	—	—
	12. L. 3	June 25	—	—	—	10	0	0	2·34	8	23·47	2	38·00	1	69·0
	,,	Dec. 25	—	—	—	8	0	0	2·51	4	21·75	2	40·30	2	67·0
	,,	May 26	—	—	—	8	1	0	2·25	8	18·95	3	35·67	2	52·0
	,,	Oct. 26	21	13	1	—	—	—	—	—	—	—	—	—	—
	3. L. 1	June 25	17	5	0	—	—	—	—	—	—	—	—	—	—
	6. L. 2	May 25	12	11	0	—	—	—	—	—	—	—	—	—	—
	Average ...	...	17·00	10·2	0·4	11·7	0·1	0·0	2·31	7·4	19·52	—	33·54	—	55·95
	Normal or alive = 10		—	10·0	0·39	10·0	0·09	0·0	—	—	—	—	—	—	—
Second First litter	42. M. 5	June 26	—	—	—	6	0	0	3·08	6	22·77	2	35·00	2	49·0
	,,	Nov. 26	—	—	—	3	0	0	3·17	3	24·93	2	22·25	1	40·0
	8. N. 2	June 26	—	—	—	13	0	0	2·13	9	14·75	1	26·00	1	33·0
	,,	Dec. 26	—	—	—	4	2	0	2·12*	—	—	—	—	—	—
	,,	May 27	—	—	—	8	0	0	2·40	6	30·10†	—	—	—	—
	,,	Oct. 27	—	—	—	4	0	0	2·95	4	22·10‡	—	—	—	—
	5. M. 1	Dec. 25	14	10	1	—	—	—	—	—	—	—	—	—	—
	46. M. 5	Dec. 25	12	11	0	—	—	—	—	—	—	—	—	—	—
	Average ...	...	13·00	10·5	0·5	6·33	0·33	0·0	2·66	5·6	22·93	—	27·75	—	40·67
	Normal or alive = 10		—	10·0	0·48	10·0	0·53	0·0	—	—	—	—	—	—	—

SECOND	24. N. 4	June 26	—	—	—	8	0	0	2·93	7	19·56	2	25·00§	—	—
Second	,,	Nov. 26	15	10	0	—	—	—	—	—	—	—	—	—	—
litter	54. P. 6	Jan. 27	—	—	—	9	0	0	2·60	6	14·92	1	15·90‖	—	—
	62. P. 6	Jan. 27	—	—	—	10	0	0	2·80	8	17·85‖	—	—	—	—
	38. N. 5	May 26	12	6	1	—	—	—	—	—	—	—	—	—	—
	45. N. 5	June 26	16	12	3	—	—	—	—	—	—	—	—	—	—
	35. N. 4	May 26	13	8	0	—	—	—	—	—	—	—	—	—	—
	7. P. 1	Dec. 26	17	15	0	—	—	—	—	—	—	—	—	—	—
	3. P. 1	Dec. 26	16	5	7	—	—	—	—	—	—	—	—	—	—
	Average		16·83	9·33	1·83	9·0	0·0	0·0	2·78	7·67	17·44	—	20·45	—	—
	Normal or alive = 10		—	10·0	1·97	10·0	0·0	0·0	—	—	—	—	—	—	—
SECOND	4. S. 1	June 27	—	—	—	10	0	0	2·75	8	31·62¶	—	—	—	—
Third	7. S. 1	July 27	—	—	—	10	1	0	2·84	7	22·91	6	27·33	5	33·20
litter	43. P. 5	Dec. 26	12	11	0	—	—	—	—	—	—	—	—	—	—
	49. P. 5	Feb. 27	11	6	2	—	—	—	—	—	—	—	—	—	—
	Average		11·50	8·0	1·0	10·0	0·5	0·0	2·79	7·5	27·26	—	27·33	—	33·20
	Normal or alive = 10		—	10·0	1·25	10·0	0·5	0·0	—	—	—	—	—	—	—
THIRD															
First litter	10. S. 2	July 27	—	—	—	4	0	1	2·82	4	22·35	4	22·12	2	33·95
	Normal or alive = 10		—	—	—	10·0	0·0	2·50	—	—	—	—	—	—	—
	Group average ...		—	9·67	1·07	9·36	0·18	0·05	2·53	6·45	20·91	—	—	—	—
	Normal or alive = 10		—	10·0	1·11	10·0	0·19	0·05	—	—	—	—	—	—	—

* All dead 2 days after birth.
† Withdrawn from experiment, July 26, 1927.
‡ Withdrawn from experiment, Dec. 24, 1927.

§ Last of litter died 55 days after weaning, Oct 12, 1926.
‖ Withdrawn from experiment.
¶ Withdrawn from experiment, Aug. 4, 1927.

Table V. *Details of protein-deficient group.*

Generation	Sow's number	Month killed or farrowed	Killed			Farrowed									
						At birth				At weaning (7 weeks)		At 12 weeks		At 16 weeks	
						Number			Av. wt. per head (lb.)						
			Ova shed	Normal	Atrophic	Alive	Dead	Atrophic		No.	Av. wt. (lb.)	No.	Av. wt. (lb.)	No.	Av. wt. (lb.)
FIRST	2. K. 1	Jan. 25	—	—	—	9	0	0	2·26	7	19·74	3	32·80	1	41·00
		Sept. 25	—	—	—	13	0	0	2·25	8	18·30	3	34·33	3	42·66
	,,	Apr. 26	—	—	—	9	0	0	2·34	5	25·08	3	37·33	3	44·66
	5. K. 2	Jan. 25	—	—	—	10	0	0	2·21	6	17·87	2	25·25	2	28·00
	,,	Sept. 25	—	—	—	15	0	0	2·10	10	15·67	3	17·67	1	23·00
	,,	Mar. 26	13	11	0	—	—	—	—	—	—	—	—	—	—
	8. L. 3	July 25	—	—	—	11	0	0	2·24	6	17·25	2	17·20	1	21·00
		Apr. 26	—	—	—	12	0	0	2·54	10	19·72	3	25·33	3	30·00
	,,	Oct. 26	—	—	—	8	0	0	2·49	4	10·47	1	19·00	1	24·80
	,,	Feb. 27	18	13	0	—	—	—	—	—	—	—	—	—	—
	11. L. 3	Aug. 25	—	—	—	5	0	0	3·12	4	23·30	1	33·00	1	37·00
		Apr. 26	—	—	—	9	0	0	3·19	7	21·59	3	22·83	2	28·00
	,,	Aug. 26	17	12	0	—	—	—	—	—	—	—	—	—	—
	2. L. 1	June 25	12	9	0	—	—	—	—	—	—	—	—	—	—
	5. L. 2	June 25	13	9	1	—	—	—	—	—	—	—	—	—	—
	Average ...	...	14·30	10·8	0·2	10·1	0·0	0·0	2·47	6·7	18·90	—	26·47	—	32·01
	Normal or alive = 10		—	10·0	0·19	10·0	0·0	0·0	—	—	—	—	—	—	—
SECOND First litter	20. M. 2	July 26	—	—	—	11	1	0	1·79	7	11·73	3	10·63	1	10·00
	,,	Feb. 27	—	—	—	10	2	1	2·38	5	19·72*	—	—	—	—
	33. M. 4	Nov. 26	—	—	—	9	1	0	2·29	5	7·82†	—	—	—	—
	97. N. 10	Mar. 27	12	11	1	—	—	—	—	—	—	—	—	—	—
	Average ...	...	12·00	11·0	1·0	10·0	1·3	0·3	2·15	5·67	13·09	—	10·63	—	10·00
	Normal or alive = 10		—	10·0	0·91	10·0	1·3	0·3	—	—	—	—	—	—	—
SECOND Second litter	125. N. 12	Nov. 26	—	—	—	12	0	0	2·22	7	14·20	3	17·67	3	18·17
	,,	June 27	—	—	—	7	0	0	3·16	7	24·21	3	36·66	3	52·66
	110. N. 11	June 27	—	—	—	4	2	0	2·40	4	21·20	4	28·00	3	31·33
	136. P. 14	Aug. 27	—	—	—	9	0	0	2·87	9	13·27	9	21·00	9	28·80
	116. N. 12	Mar. 27	12	12	0	—	—	—	—	—	—	—	—	—	—
	Average ...	...	12·00	12·0	0·0	8·0	0·5	0·0	2·66	6·75	18·22	—	25·83	—	32·74
	Normal or alive = 10		—	10·0	0·0	10·0	0·6	0·0	—	—	—	—	—	—	—
	Group average ...		—	11·0	0·29	9·6	0·35	0·06	2·46	6·53	17·71	—	—	—	—
	Normal or alive = 10		—	10·0	0·26	10·0	0·37	0·06	—	—	—	—	—	—	—

* Withdrawn from experiment, Mar. 27, 1927.

† Last of litter died, Jan. 4, 1927, 6 days after weaning.

Table VI. *Details of calcium-deficient group.*

Generation	Sow's number	Month killed or farrowed	Killed			Farrowed								
						At birth				At weaning (7 weeks)		At 12 weeks		At 16 weeks	
						Number			Av. wt. per head						
			Ova shed	Normal	Atrophic	Alive	Dead	Atrophic	(lb.)	No.	Av. wt. (lb.)	No.	Av. wt. (lb.)	No.	Av. wt. (lb.)
FIRST	1. K. 1	Jan. 25	—	—	—	8	0	0	2·17	6	22·13	1	34·0	1	44·0
	,,	July 25	—	—	—	8	1	0	3·09	7	22·79	2	44·0	2	68·5
	,,	Jan. 26	—	—	—	9	0	0	2·66	6	23·72	2	34·0	2	45·0
	,,	June 26	23	14	2	—	—	—	—	—	—	—	—	—	—
	4. K. 2	Jan. 25	—	—	—	12	0	0	2·18	6	23·40	2	48·5	2	61·0
	,,	July 25	—	—	—	6	0	0	2·27	4	28·97	2	37·6	2	56·0
	,,	Dec. 25	19	7	4	—	—	—	—	—	—	—	—	—	—
	7. L. 3	June 25	—	—	—	5	0	0	2·66	4	25·80*	—	—	—	—
	,,	Dec. 25	—	—	—	14	1	0	2·00	5	17·50	2	20·5	2	31·5
	,,	May 26	18	14	2	—	—	—	—	—	—	—	—	—	—
	10. L. 3	July 25	—	—	—	9	1	0	1·93	4	26·50	2	40·1	2	61·5
	,,	Jan. 26	—	—	—	3	0	0	2·87	3	16·27	0	—	—	—
	,,	July 26	—	—	—	10	1	0	2·28	9	13·55	2	16·9	2	21·7
	,,	Nov. 26	21	9	0	—	—	—	—	—	—	—	—	—	—
	1. L. 1	May 25	15	11	3	—	—	—	—	—	—	—	—	—	—
	4. L. 2	June 25	11	9	0	—	—	—	—	—	—	—	—	—	—
	Average ...	...	17·83	10·66	1·83	8·4	0·4	0·0	2·41	5·4	22·06	—	34·45	—	48·65
	Normal or alive = 10		—	10·0	1·72	10·0	0·48	0·0	—	—	—	—	—	—	—
SECOND First litter	28. M. 3	Mar. 26	15	12	0	—	—	—	—	—	—	—	—	—	—
	63. M. 6	Mar. 26	—	—	—	11	1	0	2·09	4	15·68†	—	—	—	—
	,,	Oct. 26	—	—	*	13	1	0	2·19	10	12·14	1	21·0‡	—	—
	,,	Mar. 27	—	—	—	11	1	0	2·51	7	19·09	3	27·93	3	36·47
	Average ...	...	15·00	12·0	0·0	11·07	1·0	0·0	2·26	7·0	15·64	—	24·46	—	36·47
	Normal or alive = 10		—	10·0	0·0	10·0	0·86	0·0	—	—	—	—	—	—	—
SECOND Second litter	77. N. 8	Aug. 26	—	—	—	3	7	0	1·73§	—	—	—	—	—	—
	,,	Feb. 27	—	—	—	13	0	1	2·77‖	—	—	—	—	—	—
	,,	June 27	—	—	—	6	3	0	3·02	4	18·78	4	21·30	4	33·38
	,,	Dec. 27	—	—	—	12	3	0	2·89¶	—	—	—	—	—	—
	79. N. 8	Aug. 26	—	—	—	12	0	0	2·19	6	8·37**	—	—	—	—
	,,	Jan. 27	17	15	2	—	—	—	—	—	—	—	—	—	—
	16. N. 3	Dec. 26	16	15	0††	—	—	—	—	—	—	—	—	—	—
	18. N. 3	Aug. 26	15	11	0	—	—	—	—	—	—	—	—	—	—
	18. P. 2	Dec. 26	11	9	0	—	—	—	—	—	—	—	—	—	—
	Average ...	...	14·75	12·5	0·5	9·2	2·6	0·2	2·52	5·0	13·57	—	21·30	—	33·38
	Normal or alive = 10		—	10·0	0·4	10·0	2·83	0·22	—	—	—	—	—	—	—
SECOND Third litter	30. P. 3	Jan. 27	—	—	—	0	2	3‡‡	—	—	—	—	—	—	—
	32. P. 3	Dec. 26	—	—	—	0	5	4‡‡	—	—	—	—	—	—	—
	,,	June 27	—	—	—	8	0	2	2·90	6	9·62	3	11·27	3	13·4
	,,	Dec. 27	—	—	—	10	2	0	2·57‖	—	—	—	—	—	—
	Average ...	...	—	—	—	4·50	2·25	2·25	2·73	6	9·62	—	11·27	—	13·4
	Normal or alive = 10		—	—	—	10·0	5·0	5·0	—	—	—	—	—	—	—
	Group average ...		—	11·45	1·18	8·3	1·32	0·47	2·44	5·7	19·02	—	—	—	—
	Normal or alive = 10		—	10·0	1·03	10·0	1·59	0·57	—	—	—	—	—	—	—

* No gilts born, so litter withdrawn from experiment at weaning.
† Last of litter died, June 1, 1926, 15 days after weaning (11·6 lb.).
‡ Last of litter died, Jan. 19, 1927, 50 days after weaning (14·5 lb.).
§ All dead 1 day after birth.
‖ All dead 2 days after birth.
¶ All dead 4 days after birth.
** Last of litter died, Oct. 25, 1926, 21 days after weaning.
†† First litter aborted.
‡‡ All dead at birth.

tinuously on a concrete floor from birth to slaughter. As twelve sows and their offspring had to be accommodated in this way, and as the capital which was granted for the original investigation, in which this rigorous confinement to concrete had not been allowed for, was insufficient to allow of as much exercise room as was properly required, the conditions in which the animals lived were not normal.

Again blood meal was used in the ration, on account of its being in practice one of the purest forms of protein, with only very small quantities of other nutrients. It is now recognised, however, to be not entirely suitable for supplying the sole protein supplement in pigs' rations. It has also to be pointed out that, both for financial and experimental reasons, one ration was fed to all pigs in a group irrespective of whether they were sucking pigs or sows. The constitution of the rations makes it clear that one could not, for this reason alone, expect anything like optimum of even average results in the early stages of each pig's existence. In spite of these disadvantages, inherent in the attempt to control very accurately the supply of protein and of mineral elements to the animals under observation, it is of importance that, on the whole, the control group gave very satisfactory results. In proportion of live pigs born, weight at birth and weight at weaning the pigs were all fairly normal. What is more important is that they remained healthy, grew normally and bred regularly.

2. As the experiment proceeded, certain tendencies in the deficient groups became more marked, and it became obvious that the total and average figures for each group over the whole period of $3\frac{1}{4}$ years would not be sufficient. The individual results have therefore been divided up according to the different generations, and in this form an analysis becomes much more interesting.

3. In this control group it will be seen that there is a steady rise in the number of atrophic foetuses from the first generation onwards. There is also a deterioration in average weight at 4 months which began to be accompanied by a lack of general condition towards the end of the investigation. This can be attributed to the very restricted exercise available, and to the absence of those factors which might have been available from grazing and rooting and which were absent from the control ration, somewhat optimistically termed "complete" at the beginning of the experiment. The weight at 4 months, however, was a very imperfect index of the maturity of the pigs in this group, because up to this time the young pigs in the complete and calcium-deficient groups, *i.e.* those pigs receiving blood meal, suffered very much from diarrhoea when

young, but later made up for the lack of growth during this period. In the protein-deficient group this diarrhoea was quite absent. It has to be noted too that the difference in weight between 7 weeks and 12 weeks is greater in the control group than in the calcium-deficient group. This is believed to be due to the fact that the control sows gave a normal supply of milk with a correspondingly normal weaning weight, whereas the calcium-deficient sows became increasingly poorer in their milk supply as shown by the weaning weights. The half-starved piglings from these sows made more relative gain when they were given adequate supplies of solid food, even though it caused diarrhoea, than the previously well-nourished piglings on the control sows. At 8 months old the control pigs were approximately normal in weight and sexually mature. There was practically no delay in breeding in this group.

(*b*) *Protein-deficient.*

1. While the obvious symptoms of calcium deficiency took some time to become fully developed, the effect of protein deficiency showed itself immediately. Reference to the 16 weeks' weight in the first generation makes this clear, and it will be seen that the full effect was achieved at once and did not increase as in the case of the calcium-deficient pigs. That this stunting was due to lack of protein was shown by the fact that access to the complete ration led very soon to normal growth. An example of this may be cited. Two pigs, litter sisters, Nos. 33. M. 4 and 34. M. 4 were 21·6 lb. and 18·8 lb. respectively when weaned at 7 weeks old on March 16, 1925. By September 7, *i.e.* when they were 225 days old (approximately 8 months) they were only 45 lb. and 46 lb. respectively. A week later 33. M. 4 was put on to the "complete" ration. In 4 weeks she showed distinct signs of oestrus, but would not accept service. On November 3 this pig had exactly doubled its weight in 57 days, and its litter sister was still only 62 lb. On February 14, 1926, the pig on the complete ration was successfully served at a weight of 230 lb., the other one being only 90 lb. Not till July 21, 1926, *i.e.* when 18 months old, was the gilt, kept on the protein-deficient ration, first served at a weight of 206 lb. From birth till 8 months old these two pigs had kept level in growth and condition, but a change on to an adequate ration of protein made an immediate difference.

2. The protein-deficiency syndrome took two clearly differentiated forms. The two pigs just referred to illustrate one of these and reference to Fig. 1 shows that partial emaciation and rough, scurfy skins were the typical symptoms. The third pig in this photograph was typical of the opposite condition, in which, although the individual was stunted for

its age, it became definitely fatter than normal, and the skin remained clean and healthy. Fig. 2 shows the same pig compared with a gilt of the same age, 8 months old, reared on the control ration. In both conditions

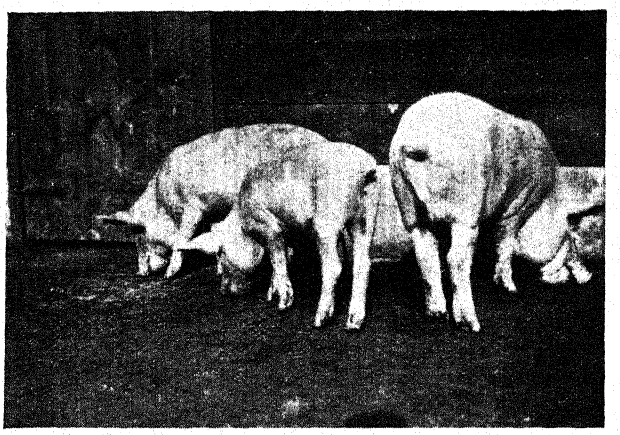


Fig. 1. Protein-deficient pigs, 34. M. 4, 33. M. 4 and 20. M. 2. Oct. 10, 1925. $8\frac{1}{2}$ months old (p. 245).

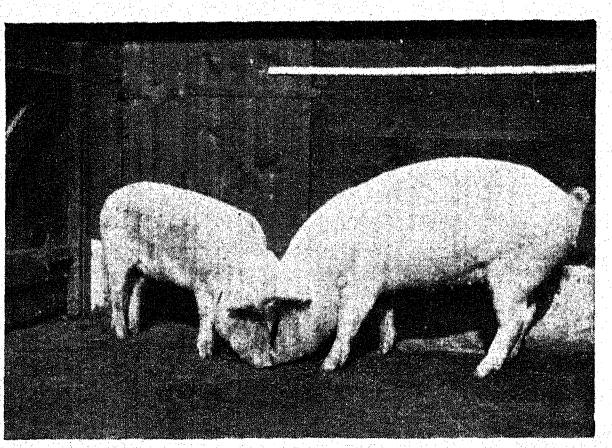


Fig. 2. Protein-deficient pig, 20. M. 2 and control pig, 5. M. 1. Oct. 10, 1925. $8\frac{1}{2}$ months old. Compare the relative size of 20. M. 2 in each case (p. 246).

the muscular development was greatly retarded, but in one case the energy derived from excess carbohydrate in the ration was dissipated in a continual restless movement such as one sees in caged wild animals, while in the other it was stored in the animal as fatty tissue. The particular syndrome depended on the temperament of the animal and this

was an inherited character. It is to be noted that other investigators, working with much smaller numbers, have on different occasions taken one condition only as being the typical one. Thus Emmett and Grindley (7) report that where two lots of four pigs each were fed on high protein and low protein rations only one pig in the low protein lot survived and a detailed examination of its carcase showed that it was definitely thinner than those from the high protein lot. Shaw (8), on the other hand, in a report of a feeding trial consisting of three groups, each of six pigs, on a complete, protein-deficient and calcium-deficient ration respectively, states that the pigs in the protein-deficient group gave carcases returning a lower price per score than those from the complete group on account of the greater fatness of the sides.

3. The most interesting observation on this group is that from an examination of the figures it will be seen that the protein-deficient ration had no effect upon foetal atrophy.

Taking first the figures for atrophic foetuses found on slaughter at 3 months, it will be seen that over the whole experiment the average number of degenerate foetuses is 0·26, taking the normal equal to 10, as compared with 1·11 in the control group and 1·03 in the calcium-deficient group. Similarly the average number of atrophic foetuses actually farrowed, 0·06, is practically the same as in the control group, 0·05. An examination of the average figures for the successive generations indicates that instead of there being any increase in atrophy there was actually less in the later stages than at the beginning.

4. Associated with these results are the figures for average number at birth, 9·6, which are just slightly higher than the control, 9·4, and the average weight at birth, 2·49 lb., which is equal to the control, 2·50 lb. On the other hand, the average number born dead, 0·37, is twice that of the control, though only about a quarter of the figure for the calcium-deficient group. Owing to the fact that the different generations in each group contain varying proportions of gilts and sows the average number of ova shed per generation is not very instructive. When the average numbers for all groups are analysed, however, the following results are obtained:

	Calcium-deficient	Protein-deficient	Control
Gilts	13·40	12·25	13·82
Sows	19·00	16·00	17·75

It is doubtful if differences of this order are significant but the figures in Table III do indicate that the proportion of the ova which develop to produce live pigs at birth is not reduced by a protein-deficient diet. The

poor results obtained by McKenzie(10) when sows were kept on a low plane of nutrition for the month following breeding are therefore not to be ascribed to a deficiency of protein.

5. A further point of interest in the protein-deficient group, not apparent from Table III but which is indicated in Table VII, is the fact that on this ration the recurrence of oestrus after weaning was very considerably retarded. Looking first at the average number of days from weaning till oestrus for each group, it will be seen that in the control group this figure is 10 days, in the calcium-deficient group 15 days, but in the case of the protein-deficient pigs it rises to 33. When this tendency was first observed it was assumed to be definitely due to the practical absence of protein in the diet. A further examination of the figures at the end of the experiment, however, suggested that this was not the case. It has already been explained that in the ration of the protein-deficient pigs a mineral mixture was to be added to take the place of the mineral elements in the blood meal fed to the control and calcium-deficient groups. Owing to certain difficulties, however, this mineral mixture was not fed during the first 20 months of the experiment. If the figures for the first generation of protein-deficient pigs are examined, it will be noted that in all cases before the minerals were added, *i.e.* those marked with a minus sign, the period is a very long one, whereas in the case of all those marked plus, indicating that they came after the addition of the mineral mixture, it will be seen that the period is in each case less than a week. This suggests that the absence of some individual mineral or combination of minerals occurring in blood meal is responsible for the slowing down of the onset of oestrus. At first sight the figures for the second generation, both first and second litters, would seem to negative this possibility, but when examined more closely, they indicate that as the experiment progressed the period became shorter. It should be explained that the sows in the second generation were from the first and second litters of the first generation, and it was just after these two litters that the abnormally long periods were experienced. It is suggested, therefore, that if the delay in the occurrence of oestrus were due to deficiency of minerals from blood meal, the second generation started life by being deprived of them in their dams' milk and that it took a considerable time to replenish the store in their bodies. In this connection it will be noticed that in the control group where the ration was approximately complete, the pre-service period was smallest, and only in this group was there a case of a sow experiencing oestrus twice whilst still suckling.

Table VII. *Periods from weaning to coming on heat.*

	Calcium-deficient		Protein-deficient		Control	
Generation	Sow	Days	Sow	Days	Sow	Days
FIRST	1. K. 1	14 –	2. K. 1	64 –	3. K. 1	13 –
	,,	5 –	,,	23 –	,,	13 –
	,,	10 –	,,	7 +	,,	8 –
	4. K. 2	5 –	5. K. 2	61 –	6. K. 2	6 –
	,,	14 –	,,	26 –	,,	16 –
	7. L. 3	14 –	8. L. 3	39 –	9. L. 3	13 –
	,,	21 –	,,	7 +	,,	19 –
	10. L. 3	9 –	11. L. 3	18 –	12. L. 3	7 –
	,,	17 – *	,,	6 +	,,	0 –
	,,	8 +	—	—	—	—
SECOND	63. M. 6	33 +	20. M. 2	84 +	42. M. 5	5 +
First litters	,,	4 +	33. M. 4	58 +	,,	6 +
	,,	5 +	—	—	8. N. 2	8 +
	—	—	—	—	,,	28 + †
	—	—	—	—	,,	5 +
SECOND	77. N. 8	59 + ‡	125. N. 12	55 +	24. N. 4	8 +
Second litters	,,	10 +	,,	16 +	54. P. 6 §	—
	,,	18 +	110. N. 11	13 +	—	—
	79. N. 8	6 +	136. P. 14	25 +	—	—
SECOND	—	—	—	—	4. S. 1	15 +
Third litters	—	—	—	—	7. S. 1	8 +
THIRD						
First litter	—	—	—	—	10. S. 2	6 +
Average –		12·2		38·3		10·5
Average +		17·9		30·1		9·9
Group average		14·8		33·58		10·2

* On heat while suckling.
‡ 7 born dead; 3 alive for 1 day.
† After dead litter.
§ Twice on heat while suckling.

(c) *Calcium-deficient.*

1. Examining first the answer to the primary question under investigation, the figures in Table VI show that in the case of the gilts and sows slaughtered in pig the proportion of degenerate to normal foetuses in the whole group, 1·03, is comparable with the control, 1·11, and in comparing successive generations the figure is actually higher at the beginning than at the end. The number of atrophic foetuses actually farrowed does however progress, and is very high in the third litters of the second generation. Owing to the high death rate and heavy casualty list in this group, it was necessary in the last stages to keep all remaining pigs for farrowing, and so no figures for slaughter at 3 months are available for this last section. Had there been any it seems probable that they would have shown a fairly high proportion of atrophy.

2. A much more definite result is seen when one studies the figures for pigs born dead but normally developed. Not only is the average for the whole group, 1·59, high as compared with the control, 0·19, but the

figures progress regularly from the first generation to the end of the second.

3. A third, and most noticeable result in this group was the apparent

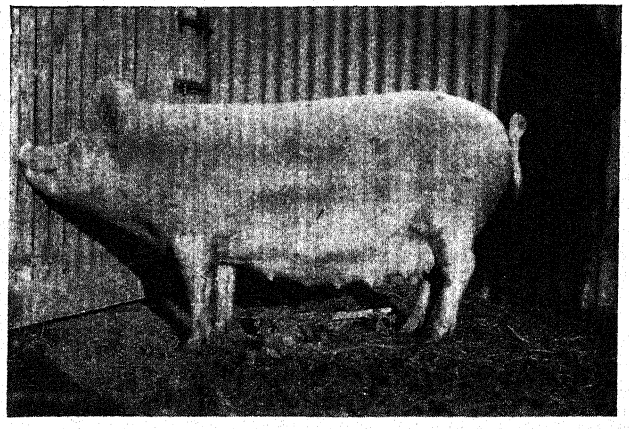


Fig. 3. Gilt on control ration, 54. P. 6. Jan. 1, 1927, 5 days before first farrowing. Although from a second litter of the third generation this gilt shows a well developed normal udder (p. 251).

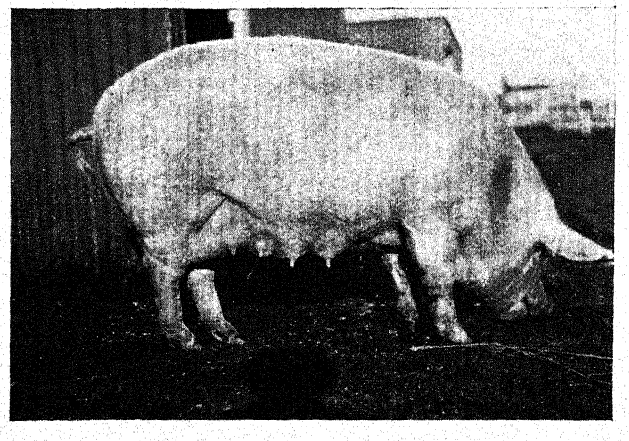


Fig. 4. Calcium-deficient sow, 63. M. 6. Nov. 9, 1926, 30 days after farrowing second litter. Note very poor development of udder when lactation was at its height, although sow only from a first litter of the second generation (p. 252).

failure of the milk supply, which became more acute with each generation. Apart from the low weights at weaning already referred to, this first became noticeable as a difficulty to draw milk from the teats just before farrowing. As the investigation continued this became more noticeable,

and there was a very marked failure in the calcium-deficient sows to develop a normal udder at farrowing time. The piglings, too, had all the appearance of being underfed and developed a very typical appearance.

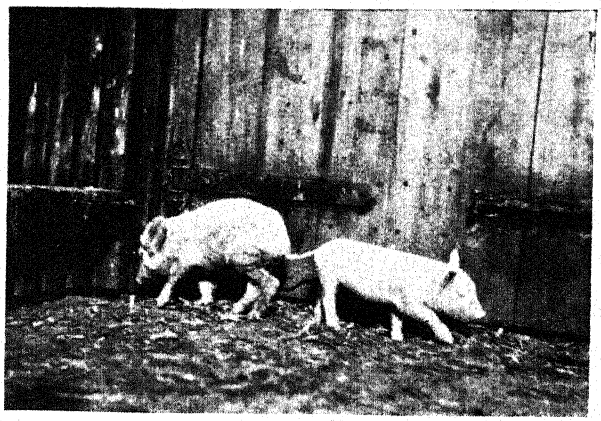


Fig. 5. Calcium deficient pigling from litter of 79. N. 8 (on left), 11 days after weaning, *i.e.* 60 days old, compared with protein-deficient pigling (on right) only 7 days old. Oct. 15, 1926 (p. 253).



Fig. 6. Calcium-deficient pigling, 87. S. 9, 60 days old, showing typical condition of piglings in this group towards the close of the experiment. Oct. 15, 1926 (p. 253).

Partly, at least, as a result of this the average weight at weaning dropped regularly with each generation, and in the later stages the pigs died within 2 days of birth, thus indicating that they probably died from complete lack of milk.

Fig. 3 illustrates a typical well-developed udder on a gilt in the control

group, and Fig. 4, showing the udder of a sow on the calcium-deficient ration, makes a very interesting comparison. This is all the more striking in that one expects to see a much greater udder development in a sow

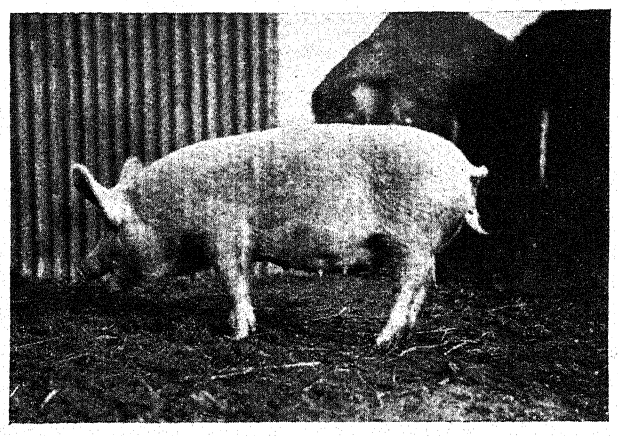


Fig. 7. Calcium-deficient gilt, 30. P. 3, on Jan. 1, 1927, 5 days before farrowing first litter. Note almost complete absence of udder development and tendency to bring the fore and hind feet together on account of stiffness of legs and weakness of back. The open mouth illustrates the squeals of pain whenever this pig was made to move about.

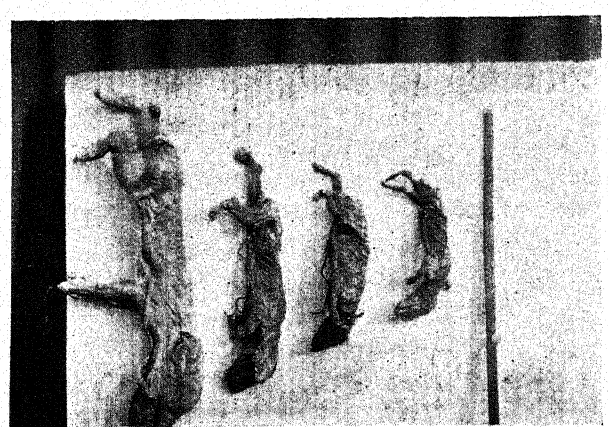


Fig. 8. Abnormal foetuses produced at farrowing of calcium-deficient gilt, 30. P. 3 (from a third litter of the second generation). One normal foetus was farrowed dead in addition to these but was destroyed in error before the photograph was taken. The gilt died 24 days after farrowing.

than in a gilt, and in the cases referred to the gilt was photographed 5 days before farrowing, *i.e.* before full development of the mammary tissue, while the photograph of the sow was taken 30 days after farrowing

when milk secretion is supposed to be at its height. These cases are quite typical of both groups in the latter part of the investigation.

Figs. 5 and 6 illustrate the extreme condition of thriftlessness which characterised the young calcium-deficient pigs when the deficiency became acute. The emaciation, coarse hair, scurfy skin, hunched back, overgrown forehead and staring protruding eye were typical symptoms in this group. It is not here claimed that this syndrome is the direct result of calcium deficiency, for it is found to occur in practice where disease of the lungs or digestive organs is present and in acute cases of infectious fevers, but the evidence points to the symptoms being secondary to rather acute starvation during the period when the piglings were solely dependent on milk, and to their being forced to eat solid food from the sow's trough before their digestive organs were capable of dealing with it.

4. Among several secondary results in the calcium-deficient group must be mentioned an abnormal number of accidents. Apart from a considerable number of animals which had to be slaughtered because of being incurably crippled, and in some cases rachitic, one pig from the first litter of the second generation (60. N. 6) broke both thigh bones as a result of slipping on the floor of its pen. Two pigs, also from the first litters of the second generation (66. M. 6 and 57. N. 6), died from peritonitis within a few days of service. It is of interest to note that in these cases the soft tissues were apparently as abnormal as the skeleton. Another pig (58. S. 7, third litter of the second generation) died from a broken spine following service, and finally a gilt (30. P. 3) (Fig. 7), also third litter of the second generation, died 24 days after farrowing two dead pigs and three atrophic foetuses (Fig. 8). No fatal accidents were experienced in either of the other groups.

(*d*) *General.*

1. Pica is a symptom very commonly observed in animals on a deficient diet. On both the deficient groups under observation it made its appearance in the second and subsequent generations. On the whole it appeared more prominently in the protein-deficient group, where it was accompanied by more restlessness and timidity than in the calcium-deficient group. When the animals were being weighed each fortnight, it was necessary to let them out of their pens for a few moments, and although they were always made to run quickly round to the weigh-bridge, it became a definitely confirmed observation that the protein-deficient pigs made an effort to obtain a bite of grass, whereas the calcium-deficient animals invariably took what chance they could of

obtaining a mouthful of soil. In the later stages of the experiment a typical symptom of the calcium-deficient pigs was the eagerness with which they hurried up to lick the pigman's boots whenever he entered the sty, although these animals were otherwise rather timid. In only two instances during the whole investigation was observation made of pigs eating dung or urine, and although three pigs in the protein-deficient group developed the habit of licking birds' droppings from the tops of the walls of the sties none of the calcium-deficient pigs was ever seen to attempt this.

2. As the experiment progressed sows in both the protein-deficient and calcium-deficient groups were observed to lose their appetite after farrowing and not to regain it till 3 or 4 weeks after weaning. The refusal to eat more than a small amount of food was accompanied by a continual nosing about in the uneaten part of the meal but in many cases the observation was made that the appetite was much improved for one or two days after the weekly bran mash.

3. Finally the numerical condition of the three groups at the end of the experiment ($3\frac{1}{4}$ years) is of considerable interest. According to the original plan there should at any given period have been in each group two sows and two gilts in pig, six store gilts ready for service and six young gilts. When the investigation came to an end in January 1928 the relative position was as follows:

The control group had bred regularly twice a year, and had reached the first litter of the third generation at the end of $2\frac{1}{2}$ years. At the close of the experiment there were two sows and two gilts in pig, five store gilts and five young gilts, a total of fourteen instead of sixteen.

The calcium-deficient group became slightly delayed in breeding and almost died out. It had only reached the third litter of the second generation at the end of three years, and all that remained were two "in pig" sows and two gilts for service. All the rest had died off or had to be slaughtered on account of acute and painful leg trouble. (See Table VI.) The breeding record of the two sows does not lead one to suppose that they would ever have replenished the group, and the steady deterioration in each successive generation leaves little doubt that the two remaining gilts would not have saved the situation. In the protein-deficient group, on the other hand, the greatest problem was that the pigs would not grow, would not breed and, paradoxically, would not even die. That is to say, that in spite of a very stunted growth and of greatly retarded breeding functions, pigs in this group did not meet with any accidents nor did they go down to infantile or other diseases. The original plan, as

already stated, assumed that all the pigs would breed fairly regularly. When, as in this case, they practically ceased growing and failed to reach sexual maturity at from 12 to 16 months old, one was forced to keep an increasing number of pigs for which there was practically no accommodation, as it was essential to get a full record of their litters alive and unborn.

The result was that at the end, although only farrowing records for the second litter of the second generation had been obtained, there were on hand three sows in pig, three gilts in pig and fourteen gilts of varying ages, none of which was likely to reach service size for a considerable number of months.

DISCUSSION OF RESULTS AND CONCLUSIONS.

Types of foetal atrophy.

The original experiment, started in 1921, and which preceded the present investigations, had as its sole object the determination of the effect of protein deficiency on foetal atrophy. At that time, the work of Evans (3) on vitamin E was not known, and the information contributed by Corner (4) has also been published during the course of the present work. It may therefore be of interest to examine the results of both these writers in comparison with those reported here.

The work of Evans was confined to rats, but the foetal condition described by him is superficially very similar to that described by Hammond and Corner. In the rat gestation always begins normally, and it is at the 11th day that characteristic symptoms appear. By the 20th day the foetus has begun to undergo autolysis, and there is grave underdevelopment of the allantois and its vessels. "Highly characteristic of this disease would appear to be the fact that the intensity and progress of these changes is different for the different implantation sites." "It is the middle germ layer and its products which in the case of the embryo seem to show the most evident alteration from the norm." Where vitamin E is completely withheld foetal degeneration takes place in all individuals, with the result that no live young are born at all. On the other hand the supply of vitamin E has both prophylactic and curative effects.

Now under conditions of normal farm practice nothing approaching this complete degree of sterility is found, and it has already been shown that the highest percentage in any one group of the present experiment did not amount to more than 5·7 per cent. In this connection, therefore,

it is suggestive that even where maximum or optimum amounts of vitamin E were supplied in Evans' experiments capable of establishing cures which were termed "complete" by the authors, there were always from 7 per cent. to 14 per cent. of resorptions. Thus the disease brought about by vitamin E starvation would appear to be something different from, or in degree very much greater than, the degeneration normally found in pigs, and for this reason its absence from or deficiency in a ration need not be considered as a cause of the foetal atrophy under investigation.

The work carried out by Corner differs from the vitamin work in that it was in the nature of a survey of the uteri from 535 pregnant sows slaughtered at two large packing houses, in New York City and Baltimore, and was not directly experimental from the point of view of nutrition. His analysis of this material indicates that the proportion of abnormal to normal foetuses was only about 2·7 per cent., but what is of greater interest is his conclusion concerning the cause of the abnormality. "Summing up these cases of pregnancy examined during the period of implantation, it seems that in the pig there is little evidence that faulty implantation is a cause of embryonic mortality and abnormality, but much to indicate that embryos may become abnormal in spite of a uterine environment which by all the criteria at our present command is anatomically and functionally normal." He further adds that he has been informed by Dr Evans in a personal communication that the placental disturbance caused by deficiency of vitamin E is due to an actual histological abnormality of the endometrium which is detectable before implantation and which is itself brought about by the dietary deficiency.

In the original investigations carried out by Hammond(1, 2) the abnormality appeared to be confined to the embryo, as was the case with the pigs examined by Corner and did not extend to the uterine tissues. Hammond also refers to a case reported by Kaufmann, who considered that with salamanders, on which he had worked, the degeneration that occurs is not due to lack of nourishment, as the relation of degenerate foetuses to the uterine wall is similar to the normal ones and moreover degenerate larvae contain quite a lot of yolk.

From a consideration of these results it would seem that there are two kinds or degrees of foetal atrophy. In the one case, so far only reported in rats, the degeneration is so far developed as to lead to complete sterility. It is caused by the adverse effect of deficient nutrition acting on the foetus through a pathological condition of the uterine tissues. On the other hand there is a degeneration of only a small portion of the total

number of foetuses varying in different cases from about 2·0 per cent. to about 20 per cent. and observed in a large number of mammals of very different kinds. In cases where only one or two are produced at a birth this type of degeneration will produce complete sterility, but is nevertheless to be differentiated from the first type.

Effects of nutrition on foetal atrophy.

The object of this investigation was to determine how far this second type of foetal atrophy might be caused by a gross deficiency of the two most important nutrients required by the growing pig, as distinct from an organic accessory factor. In the case of protein the answer is clear. Compared with the control group a deficiency of protein sufficiently acute to cause extreme retardation of growth did not lead to any increase at all.

In the case of calcium deficiency the situation is not quite so clear-cut. Reference to Table V shows that though there is practically no increase in atrophic foetuses until the very end, *i.e.* in pigs from the third litters of the second generation, the average of the four litters produced in this group was 50 per cent. However, there were only two sows in this group and of these, one was responsible for three out of the four litters, the other pig having died after its first litter. These two pigs were litter sisters, and there may have been some special factor connected with their breeding responsible for the breakdown. Nevertheless they were the only mature survivors of the calcium-deficient group, all the rest having died off or been killed on account of ill-health, and from that point of view would be those pigs which had shown the greatest individual resistance to the effects of calcium deficiency. They showed most of the typical symptoms, and it may be that the continual depletion of calcium from the lessening amounts stored in successive generations and litters reached a climax in these two pigs. This view is supported by the figures for pigs born dead or dying soon after birth.

On the other hand, if a deficiency of calcium is really responsible for the condition, why did it not appear sooner, in comparison with the control group, at a time when the other typical symptoms such as stiffness, failure of milk supply, and number of pigs born dead were all on the increase? Although it may be a contributory cause, a deficiency of calcium cannot be considered, on the evidence obtained, to be a major factor.

Effect of nutrition on oestrus.

The effect on ovulation of an increased plane of nutrition leading to a rising condition in farm animals is well known, and in this connection it is usually considered that protein-rich foods are the most suitable for "flushing." The results obtained on the protein-deficient ration suggest, however, that protein was not the determining factor in the early recurrence of oestrus after weaning. If, as is believed, the mineral mixture supplying the mineral elements of blood meal was responsible, it would suggest that either potassium or iron was the controlling factor, because the other elements were already present in ample amounts in the rest of the ration. The other possibility is that the addition of the mineral mixture led to a balance of some kind, which had a salutary effect on the nutritional economy of the animal. In this connection it has to be noted that in comparing results for the average interval between farrowings obtained under the East Anglian Pig Recording Scheme, Davidson and Duckham(11) report that in the case of one herd in which the average period was 197 days as compared with the more normal figure of 186 to 187 days found in most other herds, previous records indicated that the feeding of this herd was probably protein- and mineral-deficient.

Storage of calcium.

In the absence of the optimum amount of protein the growth of the pig is reduced to correspond with the deficiency. Observation shows that the reduction in growth also applies to the skeleton, for which presumably protein is not required in large amounts, and such functions as reproduction are retarded until a certain stage of size maturity is achieved. With calcium, however, the case is different. When this element is deficient even to a considerable degree, growth still continues in the presence of optimum amounts of protein and is only reduced very little, if at all, below the average. The result in young animals is rickets, and in older animals osteoporosis and allied pathological conditions of the bony tissues, because the body weight becomes too great for the weakened skeleton. Nevertheless the skeleton of a normal, healthy pig contains a very considerable reserve store of calcium upon which the individual can call when this element is removed from its food. Even piglings born from sows treated in this way will, especially under favourable conditions such as summer farrowing, become sexually mature without exhibiting any very acute symptoms. The small supply of milk, however, and the possibly reduced amount of calcium in the milk on which they were

reared starts them off in life with a reduced store, and their offspring in turn are usually too near the limit of anatomical and physiological requirements to be capable of successful reproduction. Under practical conditions it is unlikely that acute symptoms of lime deficiency will be observed, unless bad feeding and management is continued for some time, and the farmer will probably be unaware of the loss and danger which may be incurred from a partial deficiency. He is not so likely to be deceived by protein deficiency as the symptoms are more obvious. From a scientific point of view it may be necessary, in investigating the effect of certain nutritional deficiencies in farm animals, to ignore the results from all animals in the first generation and to consider as reliable only results from animals which, from conception, have been subjected to deficient nutrition.

Pigs born dead.

In comparison with the figures for foetal atrophy, the results of calcium deficiency as indicated by the number of pigs born dead are very definite. Table III shows that the average figure for all farrowings under this heading is 15·9 per cent. in the calcium-deficient group as compared with 3·7 per cent. in the protein-deficient group and 1·9 per cent. in the control group on the "complete" ration.

The results are even more striking when the figures are taken serially in succeeding generations and litters. In the control group this figure improved as the investigation proceeded, and in the protein-deficient group the number was highest in the middle and fell again later. In the calcium-deficient group, however, it will be seen that the consecutive figures are 4·8, 8·6, 28·3 and 50 per cent., a rapidly rising series corresponding exactly with other observed symptoms. A final reference to Table V will show that of those pigs which were alive at birth an increasing number died shortly afterwards at increasingly shorter periods.

These results, obtained under very closely controlled conditions, probably explain the striking results obtained by Baskett (5) in Northern Ireland. In the commercially managed herd reported by him, the breeding results had been getting poorer until they threatened to be a total failure. Adverse breeding factors, both in the boars and in the sows, were proved to be absent, and as the ration was deficient from a mineral point of view, a mineral supplement, rich in calcium, was added. Although the sows were allowed free access to grazing, the field in which they ran proved on examination to be excessively sour, having a lime requirement equivalent to 9·1 tons of calcium carbonate per acre. The number of pigs

born dead is not stated, but the average results per sow previous to adding the mineral supplement were:

No. farrowed	No. alive at 5 weeks	No. alive at weaning
7	3·7	2·4

At the next two farrowings, the first only a few months after the addition of the mineral supplement, the corresponding figures were:

	No. farrowed	No. alive at 5 weeks	No. alive at weaning
1st litters	10·4	7·1	7·1
2nd litters	9·7	8·1	8·1

The very poor results previously obtained appear to have been due to a gradual decrease in the calcium reserve of the brood sows caused by continual feeding on a very acid soil and on concentrates very short of calcium.

In only one case was it possible in the present experiment to put a calcium-deficient sow back on to the complete diet, 63. M. 6, after weaning her third litter, but the result did not correspond with Baskett's figures, the resultant litter containing fourteen pigs alive and two dead, of which only five were weaned in just as poor condition as the previous litters. However one case provides insufficient data for a comparison.

Effect of calcium on lactation.

Efforts extending over more than 2 years to develop a mechanical device for withdrawing milk from the sow's udder for the purpose of measurement were not successful, but close and continual observation suggested that a deficiency of calcium led to a serious reduction in the total amount of milk secreted, rather than to an appreciable lowering of the amount of calcium contained in it, though there is no confirmatory evidence to this effect in the literature of lactation in cows. In the protein-deficient group, on the other hand, there did not appear to be any very serious failure of milk supply and reference to the weights of the piglings at 7 weeks old shows that they compare favourably with the control group. In text-books and in the advisory columns of the agricultural press there is constant reference to the necessity of an adequate protein supply at the time of suckling. It would appear, however, that the necessity is really for a suitable and continuous supply throughout the whole breeding life of the animal. In the case of proteins it would appear that a shortage holds up both growth and the reproductive functions until a reasonably adequate supply has been stored. When this has

been achieved one lactation at least can be more or less successfully carried through. With calcium, on the other hand, a deficiency even of a serious degree does not hold up either growth or reproduction to any marked extent, with the result that the animal undertakes more than it is physiologically capable of, and a disastrous crisis is the result.

Sunlight and calcium metabolism.

The months in which each litter was farrowed are shown in the tables of results. In the control group the period of the year did not have a very great effect. In both the protein-deficient and calcium-deficient groups, but particularly in the latter, the best weaning results were observed in June-born litters, as will be seen from the group results, and the poorest results from farrowings in November, December and January. In the case of the protein-deficient pigs, which in some cases were thinner than normal, temperature alone may have been responsible for this result, but in the calcium-deficient pigs rickets occurred in winter, in one case (the second litter of the second generation) so severe as to necessitate the feeding of the complete ration for 3 weeks in order to carry the gilts through to service and to prevent a complete break-down of the experiment.

In the case of the pigs from the third litter of the second generation, these developed rickets in summer, but had been farrowed and weaned in the winter. Of the three main factors concerned in normal mineral metabolism, namely an adequate supply of calcium, vitamin D and sunlight, a comparison of these results with those obtained by Bohstedt and his colleagues(6) suggests that two must be present to prevent the occurrence of rickets, osteoporosis or other defects of the skeletal structure.

Blood meal.

Although blood meal caused a very troublesome diarrhoea with young pigs, it appeared to supply a fairly useful form of protein for older pigs, and the toxic effects described by Bohstedt(6) were not observed to any great extent when this food was supplemented by ground limestone, orange pulp and cod-liver oil.

SUMMARY.

(1) Previous work suggests that there are two types or degrees of foetal atrophy. In one case, so far only reported in rats, the atrophy extends to all members of a litter and produces complete sterility. This has

been shown to be connected with a pathological condition of the uterine tissues brought about by the absence of vitamin E.

Even when vitamin E is in optimum supply, however, there remains a certain amount of foetal degeneration ranging from 7 to 14 per cent. which must arise from causes other than vitamin E starvation.

In the second case a partial degree of foetal atrophy has been widely observed by different workers to occur in many different classes of mammals. Average figures for this type quoted for the pig vary from about 2 to about 20 per cent. and the residual degeneration just referred to in the rat appears to be of this nature. The present investigation was carried out to determine the effect on this second type of a deficiency in the diet of protein and of calcium. Evidence is submitted to support the conclusions which follow.

(2) Partial foetal atrophy in sows is not due to a deficiency of protein in the ration.

(3) Calcium deficiency is not the major factor in producing this type of foetal atrophy though it may be a contributory cause.

(4) None of the evidence obtained disproves the conclusions of other workers that foetal degeneration of a partial nature is probably caused by factors inherent in the zygote itself, whether these arise from lethal or sub-lethal factors or from injuries to, or malformation of the gametes.

(5) The absence of a mineral mixture representing the mineral elements of blood meal produced a very considerable delay in the return of oestrus after weaning. Potassium and iron were the only elements in this mixture not already in good supply in the ration.

(6) A ration deficient in calcium does not produce an immediate effect upon breeding sows, but the effects of the deficiency require one or two generations to become pronounced owing to the capacity to store lime in the body.

(7) Definite evidence was obtained that a calcium-deficient diet leads to a very considerable increase in the number of pigs born dead.

(8) A calcium-deficient ration leads to a very serious reduction and eventual failure of the sow's milk supply. This, combined with a large number of pigs born dead and with fatal accidents which are liable to occur, will eventually led to the extermination of a group of pigs solely confined to this diet.

ACKNOWLEDGEMENTS.

Grateful acknowledgement is made to the following for assistance during the course of the investigation: to Dr J. B. Orr, Director of the Rowett Research Institute, Aberdeen, for advice on the composition of the calcium-deficient ration; to Dr H. E. Woodman for assistance in connection with the chemical composition of the rations; to Dr James Stewart for making an analysis of the mineral elements in the blood meal used; to Dr A. Walton for assistance in recording the fertility of the boars used; and to E. A. Porter, who as pigman in charge of the experimental animals throughout, ensured the success of the investigation by constant attention to details of feeding, and loyal support during many protracted and difficult farrowings at all hours of the day and night. Reference to the support from Dr F. H. A. Marshall has already been made in the introduction.

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NOTE ON RESULTS OF THE ORIGINAL INVESTIGATION.

In the original investigation which ran from October, 1921, to March, 1924, only the effect of different proportions of protein in the ration was studied. Two groups of gilts were fed on rations which for the first 18 months had nutritive ratios of 1 : 6·87 and 1 : 4·87 and for the remainder of the experiment 1 : 5·96 and 1 : 3·51 respectively. Owing to the composition of the ration, however, there was also a very considerable difference in the proportions of calcium in each. As the pigs were all fed liberally and had access to grazing one group received a total amount of protein equal to their requirements (*vide* Table II) and the other received an excess amount of protein. No results were, therefore, obtained of the

effect of a shortage of protein, nor was the difference between the nutritive ratios sufficiently great to make the results really significant.

In so far as they went, however, the results indicated that in the pigs receiving a high proportion of protein, foetal atrophy occurred to a slightly greater extent than in the case of pigs receiving a normal protein supply, but the difference is not significant. In view of the belief that an extra amount of protein may induce "flushing" in ewes it is significant that there was no difference in the numbers of ova shed in each group nor was there any significant difference in the period between weaning and oestrus. The fact that the number of piglings born alive was slightly higher in the normal than in the high protein group is in line with the results of the main investigation though possibly insignificant.

In view of the fact that neither ration could be called extreme as regards presence or deficiency of protein, the differences in the number of live pigs born may be looked on as being due more to individuality than to ration. In this case the results clearly demonstrate that there are considerable divergences in fecundity of different strains as the differences between strains are from two to five times greater than the differences due to individuality.

(*Received December 1st*, 1929.)

INVESTIGATIONS ON YIELD IN CEREALS[1]. VII.

A STUDY OF DEVELOPMENT AND YIELD OF WHEAT BASED UPON VARIETAL COMPARISON.

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(With Plate I and Five Diagrams.)

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I. A GENERAL INTRODUCTION.

In England, the corn-growing situation, after a span of precarious years, has now become critical. Even if prices rise substantially and fairly soon, what may be described as essentially the "English cereal yield problem" will remain.

§ 1. *The English cereal yield problem.*

The present is a time of world over-production of cereals, and in particular, of wheat. Restriction of output may, before long, ameliorate the price situation but will leave the relative world position of English corn growers unchanged. A sharply limited area is at their disposal and, in world terms, their labour is costly. They are in competition

[1] The earlier papers of the series "Investigations on Yield in the Cereals," I–VI, are given in List of References, numbers 7, 8, 3, 6, 4 and 5.

with vast areas where extensive farming is economic, and also with countries of intensive cultivation where labour is relatively cheap. In England, then, output per unit area is crucial, and it must be measured monetarily. Thus, broadly, the cereal problem is to find means of increasing the national average money return per acre.

Possible means may be found both in yield and in quality. Yeoman wheat marked an immense qualitative advance; the biscuit, poultry, patent food, and domestic flour markets still hold unexhausted possibilities. But there continue to be serious economic difficulties and the importance of yield rather than quality must be regarded as paramount. Thus the cereal problem now turns upon the means of increasing average yield per acre for the whole country without substantially adding to cost of production.

It must be emphasised that the problem is a national one and, as such, must be attacked on a broader front than would be appropriate to endeavours to increase output in any one locality. The two main lines of attack are improvement of cereal varieties by breeding and improvement in the practices of husbandry.

§ 2. *External and internal yield factors.*

Agriculturally, yield or average amount of produce per unit area is a simple and definite conception. Biologically, it can be represented and discussed only as a complex which existing knowledge can but imperfectly resolve. It is an end result or integration of the vital activities of the plant throughout life. The form taken by the manifestations of these vital activities depends upon the plant's environment. To the components of environment the name "external yield factors" may be given. In a study of agricultural yield it is convenient to group the external factors as being associated respectively with the soil, the weather, the practices of husbandry, and the action of parasites. This is purely an arbitrary grouping for there is much subtle interplay between factors of the same or of different groups. Thus richness of a soil in nitrogen and soil texture, or rainfall and temperature, are well known to have important interactions. For preliminary experimentation, however, a limited number of external factors must be dealt with, their resolution into components and their interactions being deferred until further knowledge is gained. Study of external factors is an essential complement to the analysis of internal yield factors and in § 4 (*infra*) is explained the manner in which these have been approached in this series of investigations.

Every activity of the plant may conceivably exercise some influence on yield. Thus the internal factors of yield are the whole of the basal physico-chemical activities which constitute plant growth and life. They are, in fact, identical with the ultimate heritable characters or Mendelian unit characters. Every variety of a crop plant represents, of course, a separate assemblage of such unit characters. But in the existing state of knowledge, yield or Mendelian or other investigation is compelled to deal with merely the outward manifestations of these basal physico-chemical processes or unit characters. In speaking of dense and lax ears, or early and late maturation, it must be recognised that these represent complex expressions or manifestations of several true unit characters (*i.e.* activities). Moreover, the exact form of expression is directly governed by the external factors or environment.

As the true unit characters at present evade identification and measurement it is simplest to apply the term "internal yield factors" to the tangible outward manifestations which, in botany at large, go under the name of "plant characters." It is, further, convenient to speak of morphological characters, *e.g.* ear-size or tillering, and of physiological characters, *e.g.* susceptibility to frost or maturation period.

Yield analysis implies, ultimately, the identification of all the true unit characters of the separate varieties of a crop, the ascertainment of their relation to yield per unit area, and of their responses to the various external factors. As an immediate working problem it must take the form of a study of simple morphological and physiological characters under varying environments. These characters can, at present, have no absolute meaning or measurement. Their only significance is in the form of inter-varietal comparison. Thus the broad form of experimental yield analysis is inter-varietal comparison under different environments.

§ 3. *Yield analysis and plant breeding policy.*

Breeding by selection and hybridisation is many centuries old. Initially and until comparatively recent years the tendency was to cross together unusual and widely different parents in the hope of unusually valuable progeny. Experience has now shown the necessity of selecting the parent forms for specific proved merits. From combinations of these merits in subsequent hybrid generations may be obtained new and improved forms. In selecting single plants of the F_2 and subsequent generations the difficulty of identifying "high yielding capacity" is constantly met. The morphological and physiological factors of yield are not known. Consequently the only procedure is to select those plants which,

in the light of general experience, appear "promising," to propagate them into bulk, and by repeated plot trials to determine which are the highest yielders. Success turns, in large measure, upon intuitive judgment.

These same difficulties confront the breeder in selecting parent forms for crossing. Apart from general performance in cultivation there is little to indicate the specific yield merits of varieties. Thus in choice of parents as in selection from the hybrid generations procedure is entirely empirical. In theory there should be an orderly synthesis of high yielding forms, *i.e.* the systematic production by hybridisation of the most favourable combinations of internal yield factors. Only a complete analysis of yield would make this possible. Yield analysis implies, ultimately, the identification of all the internal yield factors and the measurement of their responses to environment. It lies far beyond the range of visible progress but every step taken in analysis will inform breeding with a new and less empirical spirit.

Recent history suggests that the plant breeder will be impelled into at any rate a tentative study of yield analysis. In progressive European countries the new hybrid cereal forms are superior to their predecessors mainly in quality of produce or in ancillary yield characters such as disease resistance or standing power. Their superiority in yield is often of the order of 4 to 10 per cent. under the generality of farming conditions and even this margin is not attained in all seasons. Under high farming the difference may be 12 to 15 per cent. in certain cases. Where little has been effected in crop improvement more substantial yield increases may accrue, although in India the Howards (9) find an increase of 10 per cent. to be about the maximum.

Crop husbandry is in the same case as plant breeding. There can be no direct clues to improvements in method until the biological nature of the responses of the crop to the external yield factors is better understood. Thus yield analysis, apart from its scientific attractions, thrusts itself upon the attention of all who seek to raise the national level of monetary return per acre. It is, in short, the essential investigational component of what has been described as the "English cereal yield problem."

§ 4. *The scope of investigation.*

The studies to be described in this paper represent the inter-varietal comparison aspect of the general problem of yield analysis. They are complementary to a series of experiments, already published[1], upon the

[1] See footnote on page 265.

internal factors of yield in a single variety, and upon certain external factors. A brief review of the earlier work will make clear the circumstances by which the scope of the experiments now under consideration was dictated.

Certain of the internal yield factors, with those of the external factors by which they are particularly controlled, may be regarded as indirect or ancillary. Such, for instance, are disease-resistance and ability to withstand "lodging." It has been thought desirable to exclude these from a first essay at yield analysis for they lend themselves to direct *ad hoc* investigation. Similarly the well-marked adaptations of certain cereal varieties to particular soils or climatic conditions have been relegated to a later stage of investigation. Typical instances are the eminent suitability of Little Joss wheat on very light land, of Yeoman under high farming, and the relative poorness of both on wet, heavy land where Wilhelmina is pre-eminent. Or again, in the oats, Marvellous gives extremely heavy crops in mild or even average winters. In really severe winters it fails entirely. Grey Winter Oats, on the other hand, gives medium crops even in fairly severe weather. These adaptations are of immense economic importance. Indeed the future of breeding for higher cereal yield may prove to lie in the production of specially adapted varieties. But in experiment it is exceedingly difficult to vary at will the external factors of soil and weather. Attention has therefore been mainly confined to the more controllable external factors connected with the practices of husbandry.

By these limitations the analysis of yield, as broadly outlined in § 2 (*supra*), was narrowed down to inter-varietal comparison under fairly prescribed environments. It was initiated by a comparative study of development and mature form for two barley varieties (Engledow and Wadham (7)). The object was largely to probe the difficulties of statistical sampling and to test the relation of ear-size, tillering, and certain other internal factors, to yield. Only one external factor, spacing, was studied. The essential outcome was a demonstration of the importance of spatial response and a clearer understanding of the significance of tillering. Formerly, tillering had been studied and its relation to yield sought, solely on the basis of average number of ears per plant. By following the development of tillers throughout life and the rate of their survival to the ear-bearing stage, it was possible to connect stages of development with final yield. It appeared, too, that tiller formation, apart from its connection with yield, offered a fairly direct and very simple index to plant development in general. Tiller development has,

in consequence, been adopted as the essential internal factor of yield in all the subsequent investigations.

The next step, a closer study of spatial response (Engledow(8)), brought into prominence the question of the spacing and also the other conditions, as of tilth, germination, and plant survival, in field crops. There was no existing knowledge as to these important and partly controllable external yield factors. A systematic study of typical field crops of wheat was therefore undertaken (Engledow(3)). This showed that spacing or density of plant population fluctuated in a most sharp and irregular fashion from point to point in the field. Even as between adjoining foot-lengths of a row of plants, population density was in general markedly inconstant and uncorrelated. Tillering was correlated with population density but to a degree depending very directly upon the stage of plant development. The yield of grain from short lengths of row was shown to be a direct reflection of the population density. Yield analysis upon a "field basis" proved to be readily practicable. As it removed many limitations of applicability of result to the conditions of corn-growing practice, it was made the customary method in most of the subsequent studies.

At this point experimentation was diverted to the external factors responsible for fluctuations in population density. These were shown (Engledow(6)) to lie in the seed drill rather than the tilth or the rate of plant survival. The exact mechanical defects of the drill were identified.

The inter-relations of population density, tiller development, ear formation, and yield per acre in the field, were now subjected to closer study (Doughty and Engledow(4)). Four fields, varying widely in soil and level of husbandry, but all in one locality, were developmentally compared by a survey—or "census of an acre"—of the kind already mentioned. This made patent the great influence upon yield per acre exerted by the regularity and density of plant population. It confirmed the view, steadily evolved from the earlier work, that in even the good average fields of corn in this country some 20 to 30 per cent. of the potential maximum yield was lost by reason of small gaps and local low densities in the plant population. Increased tillering and to a less degree increased ear-formation were always associated with lower population density. But the association was not sufficiently high to compensate yield per acre for paucity of plants.

The occurrence of a "critical period" in cereal plant development was established by means of these field studies. For winter corn it occurs towards the end of March and is marked by two important

features. Up to this period tillering and population density are uncorrelated. Thus the early stages of tillering are controlled by external factors other than spacing. In addition, only those tillers which have become visible by the critical period are able to survive and form ears at harvest. All others die, mainly in the months of June and July. Several important inferences arise from these two cardinal facts. It follows that within the field range of spacing, average number of ears per plant at harvest is determined by pre-critical period external factors other than spacing. On most of our wheat land, drainage and fertility—particularly as affected by nitrogen—may be expected from general experience to be important among these factors. They have been incorporated in the later stages of this general series of investigations. Again, from the general nature of the critical period phenomenon, the largest ears are to be expected from the earliest formed of the surviving tillers. Extending this supposition to the varieties of a crop, yielding capacity may be expected to depend in part on the earliness of tillering shown by the variety. General observation of many varieties has supported this deduction and earliness of tillering has already proved a valuable guide in selecting segregates from hybrid generations.

As fertility is often quickly controllable by the familiar practice of top-dressing in spring with a nitrogenous fertiliser, this external yield factor was made the next object of inquiry (Doughty, Engledow, and Sansom(5)). Tiller development throughout life was again employed as an index to growth and to the steady determination of final yield. It was shown that ordinary "spring" applications, in practice from mid-February to early April, produce increase in final yield solely by adding to ear-weight. Survival of plants and number of ears per plant are unaffected. This finding harmonises, of course, with the hypothesis of a critical period in tillering. The possibility of dual yield increase by nitrogenous top-dressing at once suggests itself. One application well before the critical period might augment ear formation; a second at the customary time might, as at present, increase ear size. Experiments to test this possibility have been in progress for two years past. It is noteworthy, too, that the impressive country-wide endeavours to raise the wheat yield of Italy represent, in effect, the application of these same inferences from the critical period hypothesis. In France improvements of similar character are under trial.

Opportunity was taken, in one of the fields, to estimate the yield loss from disease (Doughty, Engledow, and Sansom(5)). A loss of 40 per cent. from "take all" was demonstrated. The "census" method

offers a means of ascertaining the extent of loss from a number of forms of the external yield factor parasitic damage.

Side by side with the foregoing investigations there has been in progress a developmental comparison of three varieties of wheat. It has been carried on during four years and the results form the substance of this paper. The broad purpose has been to compare the varieties in respect of tiller formation and survival, ear size, and plant survival. Two spacing rates have been employed in plot work and, in addition, two of the varieties have been grown under field conditions and thus at fluctuating spacing. Inter-varietal "competition," a matter of importance in all replicated plot variety trials, has been the subject of special subsidiary experiments. The normal observations on germination speed and rate have been supplemented by special tests under circumstances representing severely unfavourable field conditions. From the meteorological records of the four years it has been possible to deduce certain broad relationships between developmental phases and weather factors.

In Section II (*infra*) will be found the details of the plots and other sowings adopted in the several experiments together with botanical and agricultural notes upon the wheat varieties employed.

II. An outline of experimental procedure.

The separate investigations are shown in tabular form in the List of Experiments (p. 273). Save for the special purposes of Exp. 5 *a–c* the seed was invariably a good sample, well matured, of about 98 per cent. germination, screened, and formalin dressed. None of the experiments was at all affected by bunt; rust, mildew, and other fungoid diseases produced no appreciable derangement; insect damage apart from slight attacks of wheat-midge (*Cecidomyia tritici, et al.*) was confined to a well-marked attack of wheat bulb-fly (*Hylemyia coarctata*) in the 1925–6 plots of Exp. 1 to which special reference is made later.

Unless otherwise stated plots were sown by hand, holes being made with the dibbing iron (described in Ref. (1)).

No special manuring, crop sequence, or cultivation was employed, the land being, in all cases except Exp. 5 *a–c* (germination under rough conditions) in a suitable state for wheat growing.

The observations throughout were developmental. That is to say, from germination to harvest periodic counts were made of number of surviving plants, number of leaves per plant (before the commencement

List of Experiments.

Experiment: No.	Experiment: Purpose	Wheat varieties used	Growing seasons	Date of sowing	Lay-out of plots, etc.
1	Varietal comparison at regular spacing	Squarehead's Master (= *A*) Yeoman (= *B*) Rivet (= *C*)	Four, viz. 1924–5, 1925–6, 1926–7, 1927–8	 Nov. 5 Nov. 5 Nov. 11* Nov. 5	8 plots of each variety arranged in chess board fashion and with competition borders; 9 effective rows per plot, 8 in. between rows, 25 seeds per row at 2 in. interval; 225 effective seeds sown per plot; on gravel soil over gault.
2	Varietal comparison under field conditions, *i.e.* at fluctuating spacing	Squarehead's Master (= *A*) Yeoman (= *B*)	One, viz. 1927–8	Nov. 3	Half-drill strip varietal trials; sown by drill in normal manner; on boulder clay over gault clay
3	Spacing experiment, all spacings being regular	Squarehead's Master (= *A*) Yeoman (= *B*) Rivet (= *C*)			
3 *a*	—	*A* and *C* only	1925–6	Nov. 15	A 4 × 4 Latin square with competition borders; each variety at two spacings, viz. 8 in. × $\frac{3}{4}$ in. and 8 in. × $1\frac{1}{2}$ in.; plots square and with 240 effective plants per plot; on a deep sandy loam at Norwich
3 *b*	—	*A* and *C* only	1926–7	Dec. 3	As for 3 *a* but at spacings of 6 in. × 1 in. and 6 in. × 2 in.; on boulder clay over gault clay at Cambridge (*i.e.* as for Exp. 2)
3 *c*	—	*A*, *B* and *C*	1927–8	Nov. 9	A 6 × 6 Latin square, *i.e.* 3 varieties at 6 in. × 1 in. and 6 in. × 2 in.; other details as for 3 *b*
3 *d*	—	*A*, *B* and *C*	1927–8	Jan. 25	Exactly as for 3 *c*, save time of sowing
4	Inter-variety competition	Squarehead's Master (= *A*) Yeoman (= *B*)	1927–8	Nov. 7	Spacing 6 in. × 2 in.; 2 seeds in every hole. In one bed varieties *A* and *B* were in alternate ranks, *i.e.* *AB*, *AB* ...; in the other, throughout the bed, one seed of *A* and one of *B* was sown in every hole; each bed contained 2400 holes, *i.e.* 4800 seeds; soil as for Exp. 1
5	Germination capacity of wheat seed under very rough field conditions	Squarehead's Master (= *A*) Yeoman (= *B*) Rivet (= *C*)			
5 *a*	—	*B* (good and bad samples) *C* (good and bad samples)	1925–6	Nov. 20	A single row 30 ft. long of good seed (laboratory germination over 98 per cent.) and bad seed (laboratory germination 70–80 per cent.) of each variety; 500 seeds per row; on very heavy, badly drained, gault clay; the land left in furrow and sown when extremely wet; furrows pulled flat with rake, drills 3 in. deep hoed out, seed sown by hand and then roughly covered in with the hoe; the top left very rough and cloddy
5 *b*	—	*B* (good and bad samples) *A* (good sample only) *C* (good sample only)	1926–7	Nov. 27	As for 5 *a* but a 4 × 4 Latin square used each "plot" in the square being a single drill 25 ft. long in which were sown 200 seeds
5 *c*	—	*B* (good and bad samples) *A* (good sample only) *C* (good sample only)	1927–8	Nov. 21	In all respects as for 5 *b*

* Sown 6 days later than usual time because of adverse soil conditions.

of tillering), number of tillers per plant (up to about the end of April), number of ears per plant, weight of grain per plant and per ear, and number of grains per ear (in Exp. 1 only). Rainfall, sunshine, and temperature data were recorded daily throughout the four seasons.

The wheat varieties used in the series of experiments were A = Squarehead's Master, B = Yeoman, and C = Rivet. All are normally winter sown. They were selected as especially appropriate to varietal comparison on a developmental basis because of their established position in English farming and their well-known biological features. Squarehead's Master exists in a number of forms, all fairly closely related. It has been grown for half a century and is still the most widely distributed wheat in England. A capacity to give at any rate a fairly good return on practically the whole range of English wheat areas is its outstanding merit. Under adversity, as of wetness of soil, or low farming, or a droughty season, it thrives more than most other English wheats. Where conditions, particularly of soil fertility, are unusually favourable, it gives increased yield but not in the same degree as many of the more modern wheats. One reason for this limitation is proneness to lodging of the straw when development is vigorous. But, apart from this, its inherent yielding capacity seems such that under high fertility it is outclassed by several other varieties over which, in turn, it is superior when conditions are unfavourable. Yeoman wheat, a well-known hybrid, is distinguished by a grain quality which transcends that of any other variety commercially suited to England, by a short and very strong straw, and by capacity to give exceptionally heavy yields under very favourable conditions. Its limitations are characteristic and interesting. To neither extreme wetness of soil nor very cold and humid climate has it the same resistance as Squarehead's Master. Moreover on light land, especially in dry years, or on any land not in good heart, it fails to do justice to itself. It is, indeed, the high-farming wheat and as such is unequalled. It will thus be seen that Squarehead's Master and Yeoman present differences appropriate to developmental comparison. Both are "common" wheats (*T. vulgare*), while Rivet is a "pollard" wheat (*T. turgidum*). This class of wheat has relatively few forms and in Great Britain is represented by Rivet alone. The agricultural characteristics of Rivet are strongly marked. Its grain quality unfits it for bread, biscuits, or domestic flour, save as a mere fraction in the miller's blend. For this reason it has decreased in cultivation but the recent demand for poultry food promises to restore it on appropriate soils. Rivet may be described as the wheat of the heaviest clays and

the wheat of convenience. It survives on heavy, water-logged land, where tillage is necessarily rough; it comes last to harvest of all the wheats; but it may safely be left standing until all the rest of the corn is up. On much of the wettest of our wheat area it alone gives a paying crop. Like all forms of *T. turgidum* it has a very woody (*i.e.* highly sclerenchymatous) straw and is thus too brittle for thatching or packing and too hard for chaff. As a contrast to the other two varieties it was thus well suited to developmental study.

It has been found convenient to denote the separate stems of the plant by the following symbols: T_0 = main axis; T_1 = first formed side tiller; T_2 = second formed side tiller, and so on.

III. COMPARATIVE DEVELOPMENT AND YIELD IN THREE WHEAT VARIETIES DURING FOUR SEASONS.

Three wheat varieties, A = Squarehead's Master, B = Yeoman, C = Rivet, were annually sown on November 5 in the four seasons 1924–8. Their familiar characteristics have been described in Section II and the plot lay-out and other details of procedure employed every year may be found in the List of Experiments (Exp. 1) on p. 273. Periodic observations were made on leaf-development and tillering. Upon the mature crop observations of number of ears per plant, number of surviving plants, ear-size etc. were made together with other analytical records. Certain evidence complementary to this is derivable from other experiments of this series. In comparing development in the three varieties it is convenient to examine the chief stages of development in turn as in §§ 1–8 which follow. All three varieties show strong fluctuations from year to year. These are discussed in connection with the crop-weather relationship in Section IX. In this present section they are dealt with only in so far as they are pertinent to inter-variety comparison.

§ 1. *Germination and plant survival.*

It has been found convenient to unite the evidence upon these points from the whole of the experiments of the series (*vide* Section VII). The salient features may, however, be recapitulated. In all three varieties 80–90 per cent. of the seeds germinate, but casualties, mostly in the first three months of life, result in a survival of seeds sown to harvest plants of 60–80 per cent. Rivet wheat shows a slightly but characteristically lower survival to harvest than the other two varieties. It is further distinguished by relative slowness of germination, a feature reflected in field as well as plot sowings, and in laboratory tests upon large numbers

of commercial samples. Late and slow germination result in a significant proportion of plants which, late in all stages of development, give under-average yield at harvest. The yield-loss so occasioned is greater in Rivet than in the other two varieties.

§ 2. *Leaf formation.*

In the initial investigations on cereal yield, characteristics of the juvenile foliage were shown to have distinct inter-variety significance (Engledow and Wadham (7), Part I). For the wheat variety studies here recorded counts were made of the number of leaves visible on the main axis (T_0) of the plant. They cover only three of the four seasons, having been omitted in 1924–5, and are set out in Table I. Leaf counts were discontinued as soon as tillering became well marked for, after that time, they naturally proved difficult. The date chosen for the first leaf count in any year was decided by the general progress of growth.

Table I. *The progress of leaf development in the three varieties*: showing average number of leaves on* T_0† (i.e. *main axis*).

		Variety		
Year	Date of count	*A*	*B*	*C*
1925–6	Feb. 4	2·09	2·59	1·96
	Feb. 18	2·60	2·75	2·40
1926–7	Jan. 20	2·18	2·75	2·00
	Jan. 27	2·72	2·90	2·34
1927–8	Jan. 21	1·83	1·93	1·83
	Jan. 27	2·01	2·46	1·94
	Feb. 3	2·39	2·87	2·37
	Feb. 11	2·86	3·03	2·76

* *A* = Squarehead's Master; *B* = Yeoman; *C* = Rivet.
† T_0 = main axis of plant; T_1 = first side tiller, etc.

It will be perceived from the table that, in an emphatic and entirely unvarying manner, Yeoman (*B*) developed its main axis leaves more rapidly than Squarehead's Master (*A*) which, in turn, was in advance of Rivet (*C*). Here, therefore, is a definite varietal feature. Its interest is enhanced by the fact of its strict analogy with rate of germination (§ 1, *supra*) and with rate of early tiller formation (§ 3, *infra*).

The year to year differences, while never overthrowing the order of the varieties, are substantial.

§ 3. *Tiller formation.*

Winter wheat, sown in practice from the end of September to the end of December, takes from three to five weeks to germinate, and first shows side tillers in a period of from ten to fifteen weeks from sowing.

The wide variation in these periods arises from differences in variety, soil, season, and tilth. Towards the end of February rate of tiller formation usually accelerates sharply, and fresh tillers continue to arise with increasing acceleration, until the end of May or even later. Tillers formed after the "critical period," which usually falls in late March, do not survive (*vide* Section I). After the end of April, even with close spacing, tillers become too numerous to allow accurate counts save by uprooting the plants. In these investigations therefore, weekly counts were made until mid-March and a final count towards the end of April. For analytical purposes it is best to record the number of side tillers (T_1, T_2, ...), *i.e.* tillers or stems additional to the main axis (T_0). This procedure has been adopted throughout. Tiller counting is necessarily arbitrary. Every visible shoot must be reckoned simply as one tiller, for it is impossible to record the size or stage of development. A statistical complication ("grouping" of observations) is occasioned by this limitation, but it does not seriously disturb results of the kind to be recorded here (*vide* Doughty, Engledow, and Sansom(5), p. 487). The actual "time of formation" of a tiller cannot, of course, be ascertained. There are some axillary buds in the embryo of the seed. It is impracticable to do other than regard a tiller as "formed" as soon as it appears above ground. Correspondingly the main axis must be treated as "formed" on the day of germination.

The tillering data are presented in Table II. Counts were made every Thursday, and the dates shown in the table apply to 1924–5: in other years counting was done on the Thursday of the corresponding

Table II. *The progress of tiller formation; showing for the three varieties the average number of side tillers per plant* (i.e. *additional to the main axis*) *and average number of ears per plant at harvest.*

Approximate date of count	1924–5			1925–6			1926–7			1927–8		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Jan. 22	0·71	0·78	0·54	—	—	—	—	—	—	—	—	—
Jan. 29	1·05	1·06	0·54	—	—	—	—	—	—	—	—	—
Feb. 4	1·38	1·68	1·18	—	—	—	0·40	0·35	0·02	—	—	—
Feb. 11	2·05	2·12	1·78	—	—	—	0·45	0·43	0·03	—	—	—
Feb. 18	2·40	2·59	2·08	0·25	0·08	0·02	0·58	0·81	0·08	0·29	0·46	0·20
Feb. 25	2·76	2·99	2·43	0·63	0·43	0·17	1·26	1·08	—	0·72	0·84	0·49
Mar. 4	3·46	3·70	3·17	0·91	0·68	0·31	1·99	2·25	1·14	1·20	1·46	1·00
Mar. 11	4·19	4·43	3·63	1·27	0·85	0·58	2·73	2·75	1·88	1·66	1·99	1·31
Apr. 22	6·60	7·09	7·87	3·40	4·04	3·80	5·73	7·67	5·97	6·84	8·85	7·71
Harvest (ears)	2·46	2·62	1·87	4·43	4·62	4·01	2·22	2·38	1·54	3·82	3·90	3·42
% of April tillers which formed ears*	32·4	32·4	21·0	100·0	91·5	83·5	33·0	27·4	22·1	48·6	39·6	39·2

* This percentage = (total ears per plant)/(1 + no. of side tillers on April 22).

week. Counting was started, in every year, well before an average of one side tiller per plant had been formed. In the whole four-year period only one count was missed—for *C* on February 25, 1927—a variety of circumstances, mainly arising from weather, having made this count impossible. Diagram I represents the same data as Table II but 1925–6

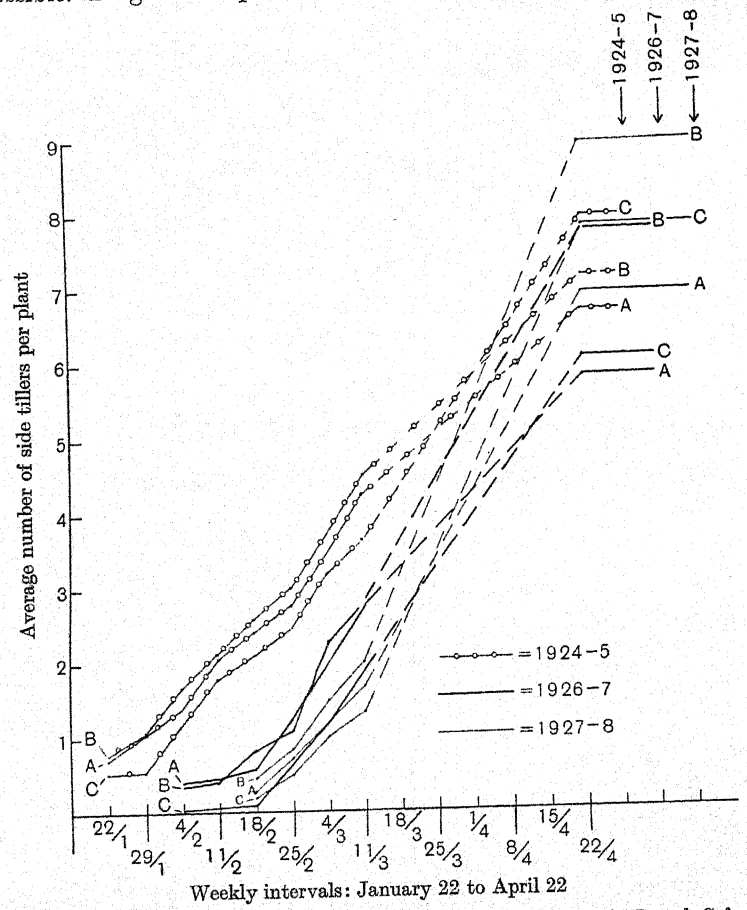


Diagram I. Average number of side tillers per plant for varieties *A*, *B* and *C* for the years 1924–5, 1926–7, and 1927–8. Date of sowing = November 5.

is excluded from it. In that year the plots were heavily attacked by wheat bulb-fly. Growth was severely checked and numerous casualties made spacing in the later stages of life extremely wide.

In all four seasons the order in which varieties commenced to tiller was *B*, *A*, *C*. A small exception will be seen in 1926–7; it disappeared

at the fourth count. Between *A* and *B* the difference is slight but significant: *C*, however, is markedly late. This same order is maintained up to March 11. Between this date and April 22 changes occur in varietal tillering rates. These may readily be appreciated from Diagram I. On April 22 the order of the varieties in respect of number of side tillers per plant is:

1924–5	...	...	$C > B > A$.
1926–7	...	...	$B > C > A$.
1927–8	...	...	$B > C > A$.

The outstanding effects in the period March 11 to April 22 are the great acceleration of tillering rate shown by *C*, the lesser acceleration of *B*, and the relative slowing down of *A*. It will be shown in later passages that these effects are reflected in the subsequent behaviour of the varieties. Their significance in relation to yield turns upon the fact that the period—March 11 to April 22—commences approximately at the "critical period" of tillering. It should be noted that the order of rapidity, $B > A > C$, shown in germination and leaf formation, is maintained in the first phase of tillering (*i.e.* up to March 11) but definitely changed in the second phase (March 11 to April 22).

The relatively high tillering rate of *A* in the first phase, and of *C* in the second phase, is shown in the frequency distribution of number of tillers per plant at the successive counts. Three examples, from 1924–5 data, are given in Table III. On March 11 the distribution for *B* was characteristically extended towards the higher values; on April 22 *B* still showed this tendency in comparison with *A*, but it was in *C* that the tendency had become most marked. The following facts, derived from Table III, further emphasise these varietal features:

On January 22 when for all varieties the average number of side tillers per plant was less than 1·0:

In *A*—66·3 per cent. of the plants had 1 or more side tillers.
B—66·3 ,, ,, ,, ,,
C—50·3 ,, ,, ,, ,,

On March 11 when for all varieties the average was about 4·0:

In *A*—37·0 per cent. of the plants had 5 or more side tillers.
B—46·8 ,, ,, ,, ,,
C—17·2 ,, ,, ,, ,,

On April 22 when for all varieties the average was about 7·0:
In *A*—35·3 per cent. of the plants had 8 or more side tillers.
B—42·3 ,, ,, ,, ,,
C—57·6 ,, ,, ,, ,,

Table III. *The frequency distribution of number of side tillers per plant for varieties* A, B *and* C *in* 1924–5 *at three periods of early tillering; frequencies are shown as percentages.*

Date	Variety	Percentage of plants having following number of side tillers																		Average no. of side tillers per plant
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Jan. 22	*A*	43·7	41·5	14·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·71
	B	33·7	54·2	12·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·78
	C	49·7	46·8	3·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·54
Mar. 11	*A*	2·3	3·7	10·5	15·3	31·2	13·3	11·2	10·7	1·8	—	—	—	—	—	—	—	—	—	—	4·19
	B	2·0	1·7	6·8	21·7	21·0	19·8	16·6	5·9	2·5	1·4	0·5	—	0·1	—	—	—	—	—	—	4·43
	C	0·5	4·7	14·0	21·4	42·1	9·1	6·0	0·9	1·2	—	—	—	—	—	—	—	—	—	—	3·63
Apr. 22	*A*	0·8	0·9	3·7	7·1	8·9	10·4	14·8	18·2	13·7	8·7	6·6	3·9	1·3	0·7	0·2	0·1	0·1	—	—	6·60
	B	0·6	0·3	1·9	4·8	7·4	13·4	14·5	14·5	14·4	10·7	7·0	4·9	2·5	1·9	0·3	0·3	0·3	—	.	7·09
	C	0·3	0·8	2·0	4·0	6·2	7·5	9·6	12·0	15·9	12·3	13·4	7·5	4·3	2·0	0·8	0·4	0·5	0·4	0·1	7·87

Among the very marked inter-year differences in tillering are few features which bear upon inter-variety comparison. Indeed the constancy of relative varietal behaviour in these widely divergent seasons is striking. There is, however, an interesting point of difference between *A* and *B* in seasonal response. It is shown by the numbers of side tillers produced in the second phase of tillering, *i.e.* March 11 to April 22, viz.:

	A	*B*	*C*
1924–5	2·41	2·66	4·24
1926–7	3·00	4·92	4·09
1927–8	5·18	6·86	6·40

The year 1924–5 was very favourable to germination and early development (up to March 11); 1926–7 and still more 1927–8 were unfavourable. These facts are plainly discernible in the times at which tiller counts were commenced and in average numbers of side tillers on March 11. The tillering data for the second phase (given immediately above) show that in 1924–5 *A* and *B* were closely similar in development apart from the characteristic but small superiority of *B*. In the other two seasons, however, *B* tillered far more than *A* in the second phase, that is, after the "critical period." These facts, supported by wide general observation, indicate the tendency of *A* to be limited in tillering after the critical period has passed, even in seasons when relatively few tillers have been formed before the critical period. The significance of this tendency depends upon the physiological status of late-formed (post-

critical period) tillers. If they merely compete with the earlier ear-bearing tillers this tendency of *A* may be of distinct importance in yield. The point is further prosecuted in the later passages of this section. Variety *C* cannot be brought into this comparison for it shows a tillering lag behind *A* and *B*. Although by April 22 it has in all years about as many side tillers as *B*, its greatest activity comes considerably later when *A* and *B* have begun to show a decline in tillering rate.

§ 4. *Ear emergence.*

Ear emergence in cereals is concentrated into a shorter time period, and is marked by far greater chronological constancy from year to year, than is the case with any other developmental plant character covered by these investigations. Throughout the earing period counts were made of the number of ears emerging every day. Emergence was taken as the stage at which the lowest spikelet of the ear had become free from the sheath of the top leaf. No marked inter-variety differences were found, save the well-known lateness of *C* as compared with *A* and *B*. The data are, in fact, of far more interest in their bearing on the influence of weather on growth than in variety comparison.

Diagram II is the record of the year 1926–7. Rivet (*C*) is definitely later than Squarehead's Master (*A*) and Yeoman (*B*), these two having very similar distributions. The asymmetry of *C* is noteworthy. Unlike *A* and *B* it shows an abrupt termination to flowering instead of a lengthy residue of late-emerging ears. The probable reason for this is the low average number of ears per plant (viz. $C = 1{\cdot}54$, whereas $A = 2{\cdot}22$ and $B = 2{\cdot}38$). In *A* and *B* many ears must necessarily have been those of late-formed tillers, which always mature a few days later than the main axis and first-formed side tillers.

In all three varieties—here as in all the other experiments of this series—the total time period of ear emergence is strikingly short, in comparison with that in which the ear-bearing tillers are formed. Germination, *i.e.* crudely, the "formation" of the main axes (T_0) of the plants, took place on December 2. The "average" plant had produced one side tiller about 70 days, and a second side tiller about 80 days after this. Thus, disregarding all later tillers, it may be said that the "formation" (*i.e.* first appearance above ground) of ear-bearing tillers covered a period of 80 days. In contrast, the ear emergence of all these tillers was compressed into a space of 20 days, or, neglecting the small proportions of very early and very late ears, into about 12 days. There is, indeed, in ear emergence, a marked "catch up" of the late tillers upon

the early formed. This same phenomenon was brought to light in a critical study of emergence dates of individual tillers in spring-sown barley (Engledow and Wadham (7), Part II, p. 72).

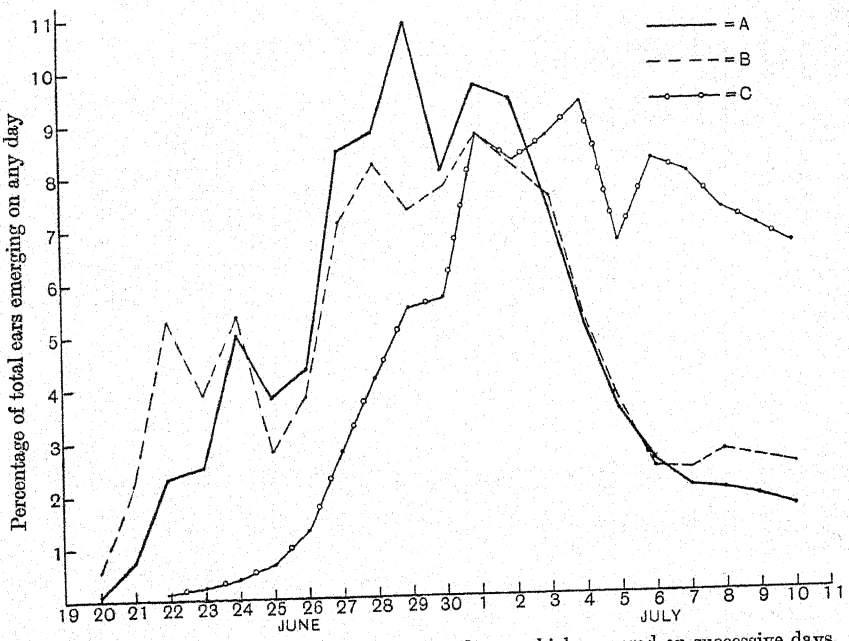


Diagram II. Showing the percentage of the total ears which emerged on successive days (1926–7 data).

There is nothing to suggest that date of flowering, *quâ* chronological phenomenon, has any direct bearing on relative varietal yield. This applies, probably, not only to the three wheats here used, but to English winter wheats as a whole. Certain indirect effects, however, are of economic importance. Thus late-flowering wheats may "sprout" in a wet harvest. But probably the most important association with flowering time is liability to attacks of wheat midge (*Cecidomyia tritici, et al.*).

§ 5. *The mature plant.*

Casualties among tillers are normally extremely heavy. Their full incidence cannot be deduced from the available data since, for reasons already given, counts ceased towards the end of April. But as the bottom line of Table II shows, the proportion of April stems (main axis plus side tillers) which survived to bear ears at harvest, varied from about 20 to 50 per cent. For the present the wholly abnormal year,

1925–6, is excluded. Varietal behaviour is characteristic for, in general form, it is constant throughout the four years. Rivet (*C*) evidently has the lowest survival rate. This harmonises, on the hypothesis of the "critical period" in tillering, with the marked tardiness of the variety in early development. Between *A* and *B* the difference is slight but characteristic, *A* having, every year, a higher tiller survival rate.

Yield per unit area and its components are given in Table IV. With the slight exception of 1926–7, Rivet always gives the heaviest yield. In general, Squarehead's Master (*A*) is second, while *B* gives the lowest yield (it is equal to *A* in 1927–8). The components shown in the table afford an explanation of the order of varieties for yield.

Table IV. *The yield attributes of varieties* A, B *and* C *in four seasons.*

	1924–5			1925–6			1926–7			1927–8		
Attributes	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Average no. of plants per plot at harvest	180·5	176·1	164·8	113·9	115·9	84·8	205·6	202·3	202·3	131·6	151·5	104·0
Average yield per plot (gm.)	764·3	617·3	823·4	583·0	479·3	826·1	977·8	813·3	957·8	916·9	918·6	1218·7
Average yield per plant (gm.)	4·23	3·49	5·01	5·13	4·13	9·79	4·76	4·02	4·71	7·35	6·06	11·60
Average yield per ear (gm.)	1·72	1·34	2·68	1·16	0·89	2·44	2·14	1·69	3·06	1·92	1·55	3·41
Weight of 1000 corns (gm.)	49·1	40·5	48·8	—	—	—	53·1	48·9	55·9	53·5	48·3	59·5
Average no. of grains per plant	86·2	86·3	102·8	—	—	—	89·7	82·2	118·8	137·4	125·5	196·0
Average no. of grains per ear	35·1	33·2	55·0	—	—	—	40·3	34·6	54·8	35·9	32·1	57·3
Average no. of ears per plant	2·46	2·62	1·87	4·43	4·62	4·01	2·22	2·38	1·54	3·82	3·90	3·42

Survival rate of plants (top line of Table IV) is very inconstant from year to year, but with the exception of 1927–8, varieties *A* and *B* are alike in survival. The superiority of *B* over *A* in this year probably explains the equality in yield of these varieties which occurs in no other year. Variety *C* shows characteristically lower survival than *A* and *B*.

In average yield per plant *C* (save in 1926–7) is superior to *A* and *B* and, in most years, very emphatically so: *B* yields definitely less than *A*. The causes governing this order ($C > A > B$) are interesting. First among them is average number of ears per plant. This is precisely as for early tillering (up to March 11), viz. $B > A > C$, and the deficiency in *C* is pronounced. It is noteworthy that experienced growers of Rivet wheat (*C*) apply seed at a higher rate (3 to 4 bushels per acre) than is their custom with other English winter wheats (2 to 3 bushels per acre).

Their practice is based, no doubt, upon the relatively low plant survival and low ear number of Rivet.

Ear weight is a second factor governing yield per plant. In this, without exception, C is markedly superior while A is always definitely above B. The importance of ear weight has, in the past few years, become plain in all cereal breeding. Grain weight (shown in Table IV as the weight of 1000 corns) also contributes to relative order of ear weight. Varieties A and C are similar with B always slightly below them. The final component of yield per plant is number of grains. As this depends upon number of ears per plant, the crucial figure is average number of grains per ear. In this the order of varieties is emphatic and constant ($C > A > B$). The great number of grains per ear formed by Rivet wheat as grown in England makes it, probably, unique among the world's wheats. Plant breeders, however, labour under the misfortune of having no really acceptable 14-chromosome wheat with which to hybridise it.

The foregoing examination may be thus summarised:

	Order of merit of varieties
Rate of germination	$B > A > C$
Germination percentage	$B > A > C$
Survival to harvest (plants)	$B = A > C$
Rate of leaf development	$B > A > C$
Rate of tillering in first phase (up to March 11) ...	$B > A > C$
Rate of tillering in second phase (March 11 to April 22)	$B > C > A$
Proportion of April tillers surviving to harvest ...	$A > B > C$
Yield per unit area	$C > A > B$
Yield per plant	$C > A > B$
Yield per ear	$C > A > B$
Number of ears per plant	$B > A > C$
Number of grains per ear	$C > A > B$
Weight of a single grain	$C = A > B$

It is thus evident that relative yield per unit area is of widely different provenance in the three varieties. The next step in yield analysis, from the point of view of variety comparison, must be to trace back the proximate manifestations of growth—tillering, ear-size, etc.—to the more immediate physiological characteristics which they represent. It will be found (Section IV *infra*) that the varietal differences described in this section find strong confirmation from the results of the entirely independent spacing experiments in which the same varieties were employed. Detailed reference to this is unnecessary here because of the closeness of agreement. Such points of disharmony as appear are indicated in Section IV. Under field conditions, as explained in Section V (*infra*), the relative varietal behaviour very closely resembles what is found on both the sets of dibbed plots.

§ 6. *Analytical notes on the mature populations.*

Analysis of the population averages given in § 5 further emphasises certain of the inter-variety differences. In Table V are shown the details of the harvest populations in the form of frequencies of plants with one ear, two ears, etc. Variety *B* has, characteristically, a slightly lower proportion of one-ear plants than *A* and slightly higher proportions of two-ear plants and of plants with five or more ears. A precisely analogous difference was shown to subsist in early tiller formation (*vide* § 3 and Table III). Correspondingly *C* differs from *A* and *B* in its high proportion of one-ear plants and in the relative infrequency of plants with three or more ears. These varietal features persist in spite of the strong year-to-year differences (*vide* Table V). Early tillering with its sequel in ear-forming capacity is thus to be regarded as a well-marked varietal character or internal yield factor.

Table V. *Showing for varieties* A, B *and* C *in the four years the percentages of plants having respectively* 1, 2, 3, ... *ears* (Roman *type*) *and the percentages of gross plot yield contributed by these groups of plants* (*italics*).

No. of ears per plant	1924–5			1925–6			1926–7			1927–8		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
1 ear	27·5	19·9	45·0	17·1	18·7	20·0	35·6	28·5	64·9	11·7	13·0	17·1
	8·8	*7·3*	*22·3*	—	—	—	*14·7*	*10·9*	*40·2*	*2·1*	*2·5*	*4·1*
2 ears	29·3	32·6	30·8	15·7	14·0	16·7	31·9	34·1	21·2	12·1	13·7	14·2
	21·6	*23·4*	*31·5*	—	—	—	*25·9*	*26·0*	*26·4*	*5·6*	*6·1*	*7·4*
3 ears	23·4	25·8	18·4	16·7	12·4	15·7	17·5	20·1	10·0	22·1	21·2	21·9
	29·5	*29·7*	*31·0*	—	—	—	*23·1*	*23·7*	*20·5*	*16·6*	*14·8*	*17·5*
4 ears	12·9	14·8	5·2	11·6	10·8	15·0	9·0	10·6	3·2	26·4	21·6	27·4
	22·5	*22·6*	*12·8*	—	—	—	*17·7*	*19·1*	*10·0*	*26·2*	*20·9*	*32·4*
5 or more ears	6·9	7·0	0·8	38·9	44·1	32·6	6·0	6·7	0·7	27·7	30·5	19·4
	17·5	*17·0*	*2·4*	—	—	—	*18·6*	*20·3*	*2·9*	*49·6*	*54·9*	*39·5*

The percentages of plot yield contributed by the one-ear, two-ear, etc., plants (Table V) are remarkable for the close similarity between *A* and *B* and the marked difference between these two and variety *C*.

The characteristics of the different ear number classes of plant are shown in Table VI. In all seasons and for all varieties the yield from a two-ear plant is more than double that from a one-ear plant, and so, correspondingly, for the higher ear number classes. It follows that as ear number increases, average ear size also increases. Thus tillering, with its culmination in ear production, is doubly an index to plant development. This conclusion has frequently arisen in the earlier investigations of this series.

The rate of increase of ear size (yield per ear in Table VI) with ear number, discloses a further difference among the varieties. It is on the

whole greatest for *A* and least for *B*; *C* is intermediate. There is a number of exceptional cases but most of these occur in the higher ear-number classes. These, being necessarily represented by relatively few plants, could not be expected to furnish very reliable evidence. In further illustration of this varietal characteristic may be noted the data of Table VII. It is there evident that the main axis (T_0) ear of the two-ear, three-ear, ... plants shows steady increase in size over the ear of one-ear plants. The swiftness of this increase is definitely in the order $A > C > B$ among the varieties.

Table VI. *Showing comparative average yield per plant, for plants having 2, 3, ... ears each, in terms of the average yield from the one-ear plants of the variety taken as* 100.

No. of ears per plant	1924–5			1926–7			1927–8		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
1 ear	100	100	100	100	100	100	100	100	100
2 ears	230	197	206	197	200	200	256	246	218
3 ears	391	316	340	322	311	331	415	356	338
4 ears	544	419	500	480	472	508	550	510	492
5 ears	798	664	649	625	672	703	750	680	665

Table VII. *Showing for two-ear, three-ear and four-ear plants the average weight of the main axis (T_0) ear in terms of the average weight of the ear of one-ear plants (for* 1924–5 *only).*

Variety	Weight of main axis (T_0) ear			
	1 ear plants	2 ear plants	3 ear plants	4 ear plants
A	100	138	159	173
B	100	120	129	142
C	100	123	143	151

It is between *A* and *B*—varieties with numerous points of general resemblance—that the differences made clear by Tables VI and VII are most marked and also most constant from year to year. Variety *B* possesses a potential yield superiority in its great capacity for early tillering which results in greater ear production. But this tends to be counterbalanced by the relative smallness of the side tiller ears compared with those of variety *A*. It is possibly upon these features that the relative field behaviour of these two varieties largely turns. Under low fertility *A* (Squarehead's Master) outyields *B* (Yeoman); they yield about equally under average conditions; but under high farming *B* is much superior in yield. Presumably it is only upon really fertile soils that *B* is able both to produce its high number of side tiller ears and to fill these ears well. In contrast, *A* always produces fewer ears, fills

its side tiller ears well even under low fertility, but is unable to show any marked response to high fertility.

Confirmation of these differences was sought by examination of tillers and ears during development. In the main axis the primordial ear begins to be formed in late March. By mid-May it is observable in all but the latest side tillers. Its degree of development may be gauged by counting the number of differentiated spikelets. To compare varieties, it is necessary to examine many plants and to draw samples with care. A basis for sampling in developmental studies was described in an earlier paper (Engledow and Wadham(7)). It consisted in using plants from the "modal" class of the tillering frequency distribution. In the tiller and primordia comparisons of *A* and *B* this method of sampling was invariably adopted. The essential results may be simply explained. Plate I—photographs of typical modal class plants—displays the characteristic varietal features of tiller size (in mid-May). Variety *A* has relatively few side tillers, fairly even in size; *B* has more side tillers, but these show marked disparity in size compared with the main axis and several of them are very small; *C* has the greatest number of side tillers, but most of them are very backward in development. Tiller size proved to be an index to development of the primordial ear. In typical plants of *A* there was a primordial ear in T_0, T_1, T_2, T_3 and T_4, and the range of development as among these was not wide. No primordial ear had commenced to form in T_5 and later tillers. Plants of *B* were quite different. Some nine stems ($T_0 \ldots T_8$) had differentiated ear primordia and development was strong in T_0; but in T_1 the primordial ear was comparatively small, while in the later tillers (T_2–T_8) it rapidly sank to extremely small dimensions. Thus the varietal difference suggested by size-range in the final ears (Tables VI and VII) was at once confirmed and explained by the facts of ear development. Plate I illustrates the principles which have been enunciated.

§ 7. *The physiological significance of side tillers.*

Tillering, the form of axillary bud development which characterises the Gramineae, is a specialised type of branching. The branches, or side tillers, arise immediately below ground level and in time develop their own adventitious root systems. The main axis of the plant also, after a dependence of varying duration upon the seminal or primary root system of the embryo, develops strong adventitious roots. A graminaceous plant is, strictly, not a unit but a colony of individuals graded in chronological age. What relationships subsist among these individuals

is not known. In connection with yield analysis the important question is the possibility of translocatory interchange among the members of a colony, *i.e.* the tillers of a plant. It seems *prima facie* improbable that such interchange should occur, but really critical tests have not as yet been devised. Differences in chronological age—it will later be shown—are considerable; it is thus probable that differences among tillers in physiological age or stage of development are such as to preclude translocatory interchange. Evidence bearing on this was adduced in an earlier paper (Engledow and Wadham(7), Part II, p. 333). It was shown that in the sequence from the main axis (T_0) down through the successive side tillers (T_1, T_2, ...) the percentage of nitrogen of the mature ear and mature straw rapidly increased. This suggests such differences in physiological state, even when all tillers are mature, as would be inconsistent with translocatory interchange.

If interchange be impossible, all those tillers which fail to form ears must be, in effect, in wasteful competition with the ear-bearing stems. Moreover, the tillers which merely form a very small ear may, by their competition with the earlier and bigger stems, actually lessen the total grain yield of the plant. It is evident then that, as 50–80 per cent. of the tillers present in May fail to survive, the exact chronology of side tiller formation may be a varietal character of some importance. As already explained it is not possible to measure nor even define the "time of formation" of a tiller. It must be regarded as "formed" on the day upon which it appears above ground. On this basis the day of formation of the main axis is the day of germination. With these postulates the chronology of tiller formation may now be examined.

Interpolation in the periodic counts of side tillers gives the day on which the "average" plant of a population had one side tiller, and so on. The process is illustrated for varieties *A*, *B* and *C*, in the season 1924–5, by Diagram III. In this the abscissae are the dates of the periodic counts commencing on 22. i (January 22), and the ordinates the average numbers of tillers per plant for the variety populations at the counts. Lines are drawn through the ordinate values, 1·0, 2·0, ... and from the points where these intersect the tillering curves perpendiculars are dropped on to the abscissa axis. Their points of intersection with this axis are the dates on which the "average" plant had 1, 2, ... side tillers. These dates are collected in Table VIII. It is seen that T_1 is formed 61 days (83 – 22) after T_0, and that T_2, T_3 and T_4 arise at periods of 12 to 16 days later still. If the "date of formation" of a side tiller, derived in the manner above, be regarded as analogous to the date of

"formation" of the main axis (*i.e.* date of germination of the seed) an interesting analogy presents itself. A sowing of variety *B* (top line of Table VIII) may be compared with a series of sowings in which every

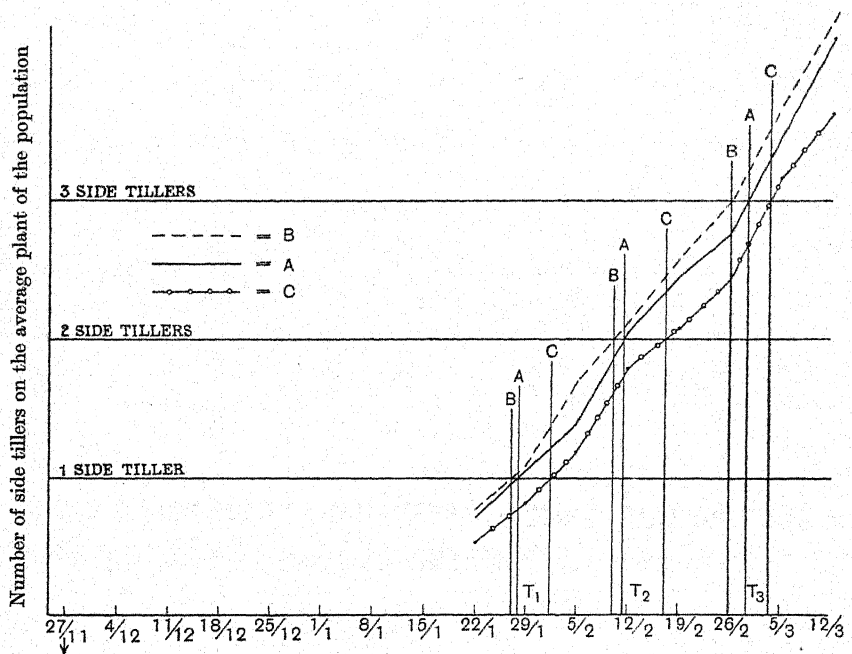


Diagram III. Showing by interpolation the dates on which the side tillers, *i.e.* T_1, T_2, T_3 were formed (1924–5 crop).

Table VIII. *Showing the dates of formation (ordinary type) of the main axis* (T_0) *and the side tillers* (T_1, T_2, T_3 *and* T_4)*; also the number of days (italics) which elapsed between sowing and the formation of the main axis and the side tillers* (1924–5 *crop*).

	Date of formation of				
Variety	T_0	T_1	T_2	T_3	T_4
B	Nov. 27	Jan. 27	Feb. 10	Feb. 26	Mar. 8
	22	*83*	*97*	*113*	*125*
A	Nov. 27	Jan. 28	Feb. 12	Feb. 29	Mar. 11
	22	*84*	*99*	*116*	*128*
C	Nov. 27	Feb. 1	Feb. 17	Mar. 4	Mar. 23
	22	*88*	*104*	*121*	*140*

plant develops only one stem. The first (true) sowing on November 5, germinates on November 27, giving a population which may be called T_0. A second "sowing"—of axillary buds as it were—"germinates" on

January 27, 61 days later, giving a population T_1. There follows a population T_2 which "germinates" 75 days (97 – 22) after T_0, and so on. This analogy, with pressing, emphasises the inevitable disadvantages of the side tiller populations as compared with the main axis. It illustrates, too, the possibility of limitation to yield per unit area arising from competition, especially in connection with late tillers which die before harvest.

The chronology of tiller development for three seasons is presented in Table IX. Data for 1927–8 are not available as weather conditions prohibited the counts required to fix the exact dates of formation of T_1. Omissions in 1925–6 and 1926–7 are similarly explainable. In general, the interval between the formation of one tiller and the next tends to decrease, *i.e.* $T_4 \sim T_3 < T_3 \sim T_2$. Invariably the interval between germination ("formation" of T_0) and the formation of T_1 is much greater than the time separating the appearances of any other pair of successive tillers. Varietal behaviour is fairly constant. The season 1925–6, in which a wheat bulb-fly attack occurred, should perhaps be neglected. In the remaining two seasons variety *B* showed a very slight advance on *A*. Rivet (*C*), in all three seasons and in respect of all tillers, was definitely in arrear of *A* and *B*. Thus tiller chronology, in the acceptation here employed, supports the subtle but important tillering differences among the varieties which have been inferred by earlier lines of reasoning.

Table IX. *Showing for varieties* A, B *and* C *in three seasons the number of days which elapsed between sowing and the formation of the main axis* (T_0)*; and between germination and the formation of the side tillers* (T_1, T_2, T_3 *and* T_4).

	T_0			T_1			T_2			T_3			T_4		
Year	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
1924–5	22	22	22	62	61	66	77	75	82	94	91	99	106	103	118
1925–6	58	58	58	63	74	80	—	—	—	—	—	—	—	110	115
1926–7	34	34	34	68	69	77	78	76	86	—	—	—	—	—	—

§ 8. *The critical period of tillering.*

Studies on field crops have demonstrated a critical period in tillering. Its characteristics, described in Section I, are briefly: up to this period spacing is without influence on tillering but subsequently is negatively correlated with it; also, tillers formed after this period die without producing ears. In field crops of winter wheat the critical period ordinarily falls at about the end of March. It is variable with seasonal and other factors. Determinations of its time incidence may be made

for the experiments here under consideration. It must be assumed that the x ears matured by a plant are borne by the main axis and the $x - 1$ side tillers first formed. Specific observation has shown this assumption to be in general true. Broadly, for all three varieties, the date of the critical period was: in 1924–5 the second week of February; in 1925–6 the third week of April; in 1926–7 the last week of February; and in 1927–8 the third week of March. These dates clearly show that when conditions of early growth are good—especially in the first three months after sowing—the critical period is early; and conversely. Its early incidence is to be regarded as a favourable yield augury, for it implies a "long physiological life" for those side tillers which ultimately bear ears. Conditions of growth subsequent to the critical period must, however, necessarily exercise a great influence on yield. Varieties *A* and *B* have, effectively, the same critical period although *B* exhibits that small advance on *A* which earlier tillering evidence would suggest. In *C* the critical period is definitely late and very early sowing—the practice of all experienced growers of this wheat—is thus plainly indicated.

IV. THE INFLUENCE OF SPACING ON THE DEVELOPMENT AND YIELD OF THE THREE VARIETIES.

Spatial response has had more attention in experimental yield analysis than any other aspect of the general problem. This has been largely because of the interest which, until a few years ago, centred upon the endeavours of Mitscherlich and others to devise a mathematical presentation of plant growth. The difficulties and dangers attending these endeavours have now been recognised (*vide* Briggs(10)). Investigation of spatial response upon a developmental basis promises to be both more safe and more informative. In developmental studies upon field crops the complexity of spacing and the directness of its influence upon yield have been outstanding (*vide* Engledow(3), and Doughty and Engledow(4)). Early on in this general series of "Investigations on Yield in the Cereals" preliminary studies of spatial response in different varieties were made on barley (Engledow and Wadham(7)) and on wheat (Engledow(8)). The closer understanding of tillering—as a developmental feature—which arose from studies of field crops, made it necessary to complement the general comparison of three wheats described in Section III, by a spatial comparison. Exps. 3 *a*, 3 *b*, 3 *c*, 3 *d* (List of Experiments, p. 273) were therefore carried out. The first, 3 *a*, included few developmental observations but gave a full analysis of the mature

population. Its results, in so far as they are relevant, support the conclusions from Exp. 3 *b–d*: they are therefore not specifically discussed in this section. In 1925–6 (Exp. 3 *a*) and 1926–7 (Exp. 3 *b*) only varieties *A* and *C* were included; in 1927–8 (Exps. 3 *c* and 3 *d*, early and late sowings) all three varieties were employed. The spacings adopted, 6 in. × 1 in. and 6 in. × 2 in. are very convenient for cereal plots and well representative of the more important part of the spatial range of the field.

§ 1. *Early development.*

In rate of leaf development the order of the varieties was precisely as in Exp. 1 (*vide* Section III, § 2), viz. $B > A > C$. But there was a curious spatial effect. In all cases, and notably in 1927–8, leaf formation was more rapid at close than at wide spacing. The average number of leaves on the main axis (T_0) was for Exp. 3 *c* (1927–8, sown November 9), at a typical count:

A		*B*	
6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.
2·26	2·14	2·82	2·79

These inter-spacing differences are statistically significant.

Tillering progress may be gauged from Table X. The varieties preserve the same order as in Exp. 1 (described in Section III), viz. $B > A > C$ in the early stages with strong acceleration in *C* at the later stages. Exp. 1 was carried out on very different soil from the spacing experiments and with different sowing dates. This constancy in the order of the varieties points to rate of tiller development as a well-marked varietal character and, therefore, internal yield factor.

Spatial influence on tillering is not very clearly brought out by the data (Table X) save in the 1927–8 early sowing. The reason lies in the unfavourable conditions at the commencement of growth in the remaining two sowings. Certain effects are, however, well marked. The first is the greater tillering at close spacing observable in the 1927–8 early sowing. It persists until a time between March 8 and March 22. Since it is so well marked it is probably not a chance effect, but, unfortunately, the data from the 1926–7 and 1927–8 late sowings start too late in the year to offer confirmation. A possible explanation is to be found in the method of sowing. Holes were made with a standard dibbing iron (described in Engledow and Yule(1), Appendix) having teeth at 2 in. apart. For the 6 in. × 1 in. spacing the iron had to be applied twice to every row, the second application making holes between those made at the first. Considerable pressure is required in using the

iron and as a result the 6 in. × 1 in. spacing must have had a "firmer seed-bed" than the 6 in. × 2 in. From what is known in practice of the advantages of a "firm bottom" for wheat, the small difference in procedure may be surmised to have influenced tillering at the outset.

Table X. *Showing average number of side tillers per plant for varieties* A *and* C *in* 1926–7 (*sowing date Dec.* 3); *for* A, B *and* C *in* 1927–8 (*sowing date Nov.* 9); *and for* A, B *and* C *in* 1927–8 *late sowing* (*sowing date Jan.* 25); *in all cases at two spacings, viz.* 6 *in.* × 1 *in. and* 6 *in.* × 2 *in.*

Variety	*A*		*B*		*C*	
Spacing	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.
Date of count			1926–7 (sown Dec. 3).			
Mar. 14	0·51	0·53	Not included		0·09	0·14
Apr. 4	2·02	2·35	,,		1·61	1·88
Apr. 16	2·66	3·78	,,		2·32	3·12
Apr. 23	2·99	4·67	,,		2·74	4·35
May 2	3·08	5·01	,,		2·86	4·89
			1927–8 (sown Nov. 9).			
Feb. 16	0·25	0·17	0·72	0·60	0·03	0·02
Feb. 23	0·72	0·55	1·12	0·96	0·57	0·47
Mar. 1	1·15	1·03	1·77	1·61	0·83	0·76
Mar. 8	1·81	1·67	2·52	2·43	1·55	1·46
Mar. 22	3·07	3·19	3·95	4·15	2·64	2·56
Apr. 10	3·63	5·26	5·21	7·66	4·69	5·42
			1927–8 (sown Jan. 25).			
Apr. 11	1·87	1·85	2·55	2·86	—	—
May 2	3·19	5·17	3·95	6·77	4·11	5·05

In all sowings wider spacing ultimately induced greater tillering. Its extent and its bearing on yield are discussed in § 3 (*infra*). The time at which spatial response to tillering became apparent shows interesting variations as between varieties, spacings, and sowings. Their significance is examined in connection with the critical period of tillering in § 4 (*infra*).

§ 2. *Ear emergence.*

The comparative constancy of date of ear emergence in cereals, and date of flowering in many other plants, is well known. Percival(11) gives striking examples. Thus, he found (pp. 86 *et seq.*) for Red Fife wheat at Reading in 1912–13:

Date of sowing	Date of ear emergence
Sept. 7	June 7
Oct. 5	June 7
Dec. 7	June 12
Feb. 24	June 15
Mar. 2	June 17

In these spacing experiments the dates were:

	Date of sowing	Date of ear emergence		
		A	*B*	*C*
1926–7	Dec. 3	June 20	(Not sown)	June 24
1927–8	Nov. 9	June 21	June 22	June 27
1927–8	Jan. 25	June 27	June 27	July 5

These dates apply to both spacings, for the difference between them was so slight as to escape observation. The small delay in ear emergence occasioned by the very late sowing in 1927–8 is noteworthy.

§ 3. *The mature plant.*

Ear formation and its relation to early tillering are shown in Table XI. In this, as a basis of estimation of tiller survival, the amount of tillering on April 10 is adopted. This appears to be the most suitable date but it does not apply, for the 1927–8 late sowing, to variety *C* (Rivet) in which tillers continued to form rapidly until late in May. At the wide spacing there is always a greater number of ears per plant than at the close, although the ratio never becomes as high as 2 : 1. Varieties *A* and *B* show about the same increase in ear formation as between the two spacings; in *C* the increase is always less.

Table XI. *Showing for* A, B *and* C *at three sowings: column* (1) *Average number of stems per plant* (*side tillers* plus *main axis*) *on April* 10*; column* (2) *Average number of ears per plant at harvest; column* (3) *Ratio* (*per cent.*) *of ears to April stems*, i.e. *column* (2)/*column* (1).

	1926–7 (sown Dec. 3)			1927–8 (sown Nov. 9)			1927–8 (sown Jan. 25)		
Variety and spacing	Average no. of stems per plant on Apr. 10 (1)	Average no. of ears per plant (2)	Ears/Stems % (3)	Average no. of stems per plant on Apr. 10 (1)	Average no. of ears per plant (2)	Ears/Stems % (3)	Average no. of stems per plant on Apr. 10 (1)	Average no. of ears per plant (2)	Ears/Stems % (3)
A. 6 in. × 1 in.	3·50	1·20	34	4·63	1·29	28	2·87	1·44	50
6 in. × 2 in.	4·50	2·10	47	6·26	2·17	35	2·85	2·41	84
Difference between spacings (as % of lower)	29	75	13	35	68	7	0·0	67	34
B. 6 in. × 1 in.	—	—	—	6·21	1·45	23	3·55	1·48	42
6 in. × 2 in.	—	—	—	8·66	2·43	28	3·86	2·50	65
Difference between spacings (as % of lower)	—	—	—	39	68	5	9	69	17
C. 6 in. × 1 in.	3·10	1·06	34	5·69	1·33	23	—	1·63	—
6 in. × 2 in.	4·00	1·67	42	6·42	2·00	31	—	3·10	—
Difference between spacings (as % of lower)	28	58	8	13	50	8	—	90	—

The proportion of April tillers which survived to form ears is shown in columns (3) of Table XI. In all seasons and for all varieties the proportion is greater for the wider spacing. This is in conformity with results from field crops (Engledow (3), p. 188), for which the data were:

Average number of plants per foot	6·0	11·0	20·0
Average number of tillers per plant (in May)	5·9	4·8	3·8
Proportion of May tillers which formed ears (%)	42·4	38·4	34·2

The increase in percentage survival of tillers as between the two spacings is greater for variety *A* than for *B*. Variety *C* cannot be brought into the comparison because of its lateness in tillering (as explained above). On the whole, for both spacings and for all three sowings, the order of tiller survival rate among varieties is $A > B > C$.

The constitution of the mature populations is shown in Table XII. Variety *B* is omitted because of its close similarity to *A* and for 1926–7 no yield data are given, for the plots in that year were damaged by birds in the week preceding harvest. A marked difference between *C* and the other two varieties is apparent in the ear-forming capacity displayed at both spacings.

Table XII. *Showing for varieties* A *and* C *in three sowings the percentages of plants having respectively* 1, 2, 3, ... *ears* (Roman *type*) *and the percentages of gross plot yield contributed by these groups of plants* (*italics*); *variety* B *closely resembles* A *and the data from it are therefore omitted:* (*for* 1927–8 *early = sown Nov.* 9; *late = sown Jan.* 25).

No. of ears per plant	*A* (6 in. × 1 in.)			*A* (6 in. × 2 in.)			*C* (6 in. × 1 in.)			*C* (6 in. × 2 in.)		
	1926–7	1927–8 early	1927–8 late	1926–7	1927–8 early	1927–8 late	1926–7	1927–8 early	1927–8 late	1926–7	1927–8 early	1927–8 late
1 ear	82·2	77·5	62·5	29·9	33·0	24·4	94·6	73·0	51·0	47·0	35·2	13·5
	—	*58·5*	*43·0*	—	*14·4*	*8·3*	—	*55·3*	*28·2*	—	*17·0*	*3·2*
2 ears	15·9	16·7	30·8	36·6	30·6	29·4	5·2	21·4	38·4	39·9	36·6	23·6
	—	*27·4*	*43·0*	—	*28·6*	*25·4*	—	*32·6*	*49·4*	—	*35·6*	*13·6*
3 ears	1·9	4·6	6·6	27·0	25·8	30·6	0·2	5·1	10·4	12·2	22·4	31·4
	—	*10·2*	*14·0*	—	*35·3*	*38·5*	—	*11·0*	*20·0*	—	*33·4*	*28·8*
4 ears	—	1·3	—	6·1	8·2	11·5	—	0·4	0·9	0·9	5·1	21·4
	—	*3·9*	—	—	*15·6*	*19·7*	—	*1·1*	*2·9*	—	*11·5*	*31·6*
5 ears	—	—	—	0·4	1·7	3·6	—	—	—	—	0·4	4·5
	—	—	—	—	*3·7*	*7·7*	—	—	—	—	*1·8*	*7·5*
6 ears	—	—	—	—	0·4	0·2	—	—	—	—	0·2	5·6
	—	—	—	—	*1·2*	*0·3*	—	—	—	—	*0·7*	*15·3*
7 ears	—	—	—	—	0·3	—	—	—	—	—	—	—
	—	—	—	—	*1·1*	—	—	—	—	—	—	—

The two spacing populations show, for every variety and in all seasons, sharply different constitutions, *i.e.* proportions of plants with one ear, two ears, etc. The magnitude of these calls for special notice in

later passages where the influence of spacing upon ear formation in field crops is discussed.

Differences in tillering, with their outcome in ear formation, underlie the differences in yield and yield attributes associated with variety and spacing. A conspectus of yield data is afforded by Table XIII (1926–7 results are excluded because of damage by birds). The marked variation throughout the table between the early and late sowings of 1927–8 is remarkable. It is believed (*vide infra*) to be an effect of season, for weather conditions in the first three months after sowing were such as to outweigh the disadvantages which, in most seasons, would arise from late sowing.

Table XIII. *The yield attributes of varieties* A, B *and* C *at two spacings and two sowing dates in* 1927–8.

	1927–8 early (sown Nov. 9)						1927–8 late (sown Jan. 25)					
	A		*B*		*C*		*A*		*B*		*C*	
Attributes	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.
Yield per unit area (gm.)	244·0	221·9	211·5	216·4	292·9	269·9	202·6	234·4	178·9	213·6	377·7	441·5
Average yield per plant (gm.)	1·90	3·46	1·69	3·15	2·97	5·52	2·13	4·50	1·68	3·36	4·39	9·92
Average yield per ear (gm.)	1·45	1·59	1·17	1·30	2·24	2·76	1·48	1·87	1·14	1·34	2·69	3·20
Weight of 1000 corns (gm.)	51·90	51·83	45·70	45·70	55·50	55·47	54·05	54·02	47·90	47·86	58·25	58·25
Average no. of grains per plant	38·5	66·7	37·0	68·9	53·5	99·5	39·4	83·3	35·1	70·2	75·4	170·3
Average no. of grains per ear	27·9	30·6	27·8	28·4	40·4	49·7	27·5	34·6	23·8	28·0	46·2	54·9

The essential features of yield per unit area may be examined first. For both sowings and at both spacings the varietal order is $C > A > B$. In one or two cases (*e.g.* early sowing, *A* and *B*, 6 in. × 2 in.) the variety difference is not statistically significant but, in general, the order is emphatically as stated above. Thus these plots, on different soil and with different sowing dates, afford a striking confirmation of the relative yielding capacities of the varieties as measured in the entirely independent experiments described in Section III.

Spacing exercised only a slight influence on yield per unit area in the early sowing, there being merely a suggestion of greater yield at the closer spacing. For the late sowing, however, the wider spacing gave, in all three varieties, an increase in yield of some 18 per cent. "Season" has thus outweighed the influence of spacing. In both yield per plant and yield (*i.e.* average weight) per ear, the order of varieties at both

spacings and for both sowings is $C > A > B$. This, again, confirms the results recorded in Section III. Extent of ear formation, however, gives results at variance with those of Section III. In place of the order $B > A > C$ (Exp. 1) Table XIII shows, for the early sowing $B > C > A$, and for the late $C > B > A$. No great importance attaches to this disharmony between the two experiments which, on grounds already explained, is believed to be no more than a very exceptional seasonal effect.

The influence of spacing upon the yield attributes must now be examined for the varieties in turn. Yield per plant is, for *A*, *B* and *C*, roughly twice as great at the 6 in. × 2 in. spacing as at the 6 in. × 1 in. in the case of the early sowing (Table XIII). For the late sowing it is significantly more than double in variety *C* and about double in *A* and *B*. Wider spacing has a well-marked effect on average ear size (yield per ear in the table) in all cases. Thus, with increase in spacing, the plant forms more ears and larger ears, an effect corresponding with concurrent rise in ear number and in ear size among the plants of any one population (cf. Section III). For varieties *A*, *B* and *C* the response of ear size to spacing is of about the same order.

Grain size (weight of 1000 corns in Table XIII) shows no response to spacing, but is definitely greater for the late sowing as a whole than for the earlier. Since for each sowing, grain size is unaffected by spacing, the influence of spacing upon ear weight must be connected with number of grains per ear. This is apparent from the bottom line of the table. In all cases wider spacing has induced the formation of more grains per ear.

The essential influences of spacing upon yield appear, therefore, to be reflected in ear formation and grain formation. In these experiments the effects are, briefly:

At the 6 in. × 2 in. spacing, in comparison with the 6 in. × 1 in. the increase in average number of ears per plant is:

For variety *A*—about 70 per cent. of the 6 in. × 1 in. value.
B— ,, ,, ,, ,,
C— ,, 55 per cent.

The corresponding increases in average number of grains per ear are:

For variety *A*—10 and 25 per cent. for the early and late sowings.
B—25 and 17 ,, ,, ,,
C—23 and 19 ,, ,, ,,

These results are important. Yield increase per unit area may be brought about, proximately, by circumstances which augment any of the following—survival rate of plants, number of ears per plant, number of grains per ear, and grain size. Actually, of course, most circumstances which affect one of these attributes, affect one or more others also. But, in studies of the influence of nitrogenous fertilisers, it has been found that, of the four attributes, grain size is most readily influenced and number of grains per ear least readily. It appears then—at any rate under plot conditions—that the "spacing" factor exercises upon yield attributes influences which are possibly distinctive.

As a whole the inter-varietal differences in response to spacing, here described, are not well marked. In an earlier experiment (Engledow(8)), where Yeoman and Red Fife wheats were compared at a range of spacings, strong variety differences appeared. These two wheats, however, are widely divergent in field behaviour, Red Fife rarely giving an economic yield under English conditions. It appears probable, from the comparison of Squarehead's Master (*A*), Yeoman (*B*), and Rivet (*C*) that, in the inter-variety comparison aspect of yield analysis, the spatial factor will call for little more attention. Its importance in the study of external yield factors is, on the other hand, quite evident. At spacings of 6 in. × 1 in. and 6 in. × 2 in. the three varieties have given more or less equal yields. In spite of this, spatial fluctuation in field crops has been shown to exercise a marked influence on yield per unit area. Discussion of this paradox is deferred to Section V.

§ 4. *The critical period of tillering.*

The hypothesis of a critical period of tillering was described in Section I. It was originally deduced from studies on field crops by the "census" method. Two properties attach to the period: (α) it is only the tillers formed before the critical period which survive and form ears; (β) up to the critical period spacing does not influence tillering. In a field crop there are many gradations of spacing and, for these unitedly, the two properties mentioned above are virtually synchronous and so constitute a critical period. The chronology of the period for field crops varies somewhat from year to year. Moreover, in Section IV, § 8, it has been shown that on plots the time incidence of the limitation of ear-bearing tillers may be inconstant. Thus, in the four seasons of 1924–8 for Exp. 1, it varied from the second week in February to the third

week in April, although sowing date and spacing were the same in all years. The spacing experiments which have been described make it possible to examine the time variations in the second property of the critical period, namely, spatial influence upon tillering. These variations are well marked. Taken in conjunction with the time variations of the first property, they cast a new light on the yield significance of the critical period.

In Table XIV are given the full data of the time incidence of the two properties of the critical period for the three wheat varieties at two spacings, and in three separate sowings. The first property (α) (limitation of ear-bearing tillers) must be examined for response to spacing, to variety, and to season.

Table XIV. *Showing time of occurrence of the two phases of the critical period of tillering, viz.* (α) *Period before which tillers must be formed in order to produce ears;* (β) *Period before which spacing does not influence tillering. The unit of time is a week and the weeks of the month are denoted by* I, II, ...

	A		*B*		*C*	
	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.
Phases of the critical period	1926–7: sown Dec. 3					
(α) Period before which tillers must be formed to produce ears	March II	March III	—	—	March II	March IV
(β) Period before which spacing does not influence tillering	March I		—	—	March III	
	1927–8: sown Nov. 9					
(α) Period before which tillers must be formed to produce ears	Feb. III	March I	Feb. III	March I	Feb. III	March I
(β) Period before which spacing does not influence tillering	March III		March II		March IV	
	1927–8: sown Jan. 25					
(α) Period before which tillers must be formed to produce ears	March III	April I	March III	April I	March III	April I
(β) Period before which spacing does not influence tillering	April II		April I		April III	

The response to spacing is definite and almost constant, for in the 6 in. × 2 in. sowings the limiting time for formation of ear-bearing tillers is in all cases save one (variety *A*, 1926–7) a fortnight later than in the corresponding 6 in. × 1 in. sowings. This result is to be expected, in so far as the number of tillers surviving to form ears is controlled by inter-plant competition. The wider the spacing the longer the competition-free period. Freedom from competition is likely, also, to favour

tiller size; and it will be recalled that, as spacing increases, both ear number and ear size in the mature plant show increase. A question of obvious practical importance is whether, by timely use of fertilisers or in any other way, it is possible to secure for closely sown plants, the advantages which arise from wide spacing. To answer this will involve a study of the nature of inter-plant competition in early life. Presumably it operates through increasing shortage of one or more of the essentials of plant life. Experiments now in progress make it doubtful whether available soil nitrogen—which naturally suggests itself—is the chief deficiency factor.

As among the varieties there is virtually no variation in the time limitation to the formation of ear-bearing tillers. This holds good at both spacings and in all three sowings. It follows then, for these three varieties, that the index to ear production per plant is rate of early tillering. General experience, especially in selecting segregate forms in hybridisation, suggests that this index has wide application among wheat, barley and oats, and is a valuable guide in breeding.

The influence of "season" (either the year or the sowing date) is apparent from Table XIV. Favourable early conditions bring the limitation to formation of ear-bearing tillers into operation at an early date. Precisely the same conclusion was reached from the experiments described in Section III. In § 8 of that section the advantages in final yield which may accrue from early incidence of this property or phase of the critical period have already been explained.

Consideration must now be given to time variations in the second property of the critical period (in Table XIV (β) Period before which spacing does not influence tillering). Here, again, season induces sharp effects. For all three varieties the time incidence is 1 to 2 weeks later for 1927–8 early sowing than for 1926–7, and 3 weeks later for 1927–8 late sowing than for 1927–8 early sowing. It will be perceived, by referring to Table X, that the three sowings do not show the same chronological order for incidence of this second property of the critical period as for date of sowing, or for rate of early tillering. Thus the 1927–8 early sowing was sown before the 1926–7 and tillered far earlier; but the critical period (for spacing influence) was a week later in variety *C* and 2 weeks later in *A*.

The second (spatial influence) property—unlike the first (limitation to ear-bearing tillers)—displays characteristic inter-variety differences.

These may be thus summarised (here the sign $<$ implies "earlier than"; $>$ implies "later than"):

In 1926–7	A 2 weeks $< C$.
In 1927–8 early sowing	B 1 week $< A$;
	B 2 weeks $< C$.
In 1927–8 late sowing	B 1 week $< A$;
	B 2 weeks $< C$.

These differences correspond with the order of early tillering (*vide* Section III, § 3 and Section IV, § 1) which was $B > A > C$ (*i.e.* B tillers earliest, C latest).

The essential conclusions which have been reached as to time incidence of the critical period are, therefore:

(i) Properties (α) and (β) of the period are not always synchronous.

(ii) Season sharply affects the time incidence of both.

(iii) While (α) shows no inter-variety differences, such differences are well marked for (β), the order of earliness of incidence being $B < A < C$.

From (iii) it follows that, while the time limit for formation of ear-bearing tillers is the same for all varieties, the limiting effect of spacing on tillering is felt soonest in B, the variety that by tillering earliest produces most ears per plant at harvest. Now tillering has proved to be an index to general vigour of growth. Thus, it is in B that growth is first restricted by spatial influence, *i.e.* inter-plant competition. Consequently the size of the side tillers and the size of the ears they bear, should be in B relatively smaller than in A, and in A relatively smaller than in C. This is precisely the result shown by analysis of the harvest data in Exp. 1 (*vide* Section III, § 6). It must, therefore, be recognised that very early tillering varieties, while producing a high average number of ears per plant at harvest, inevitably tend to produce relatively small ears on their side tillers. The limitation thus imposed upon breeding for higher yield is patent. In principle, it can be overcome only in one way—by high farming. Provided the season be not definitely adverse and if, at the onset of the inhibiting effect of spacing, soil conditions are such as to promote strong growth in the first-formed side tillers, both numerous and relatively good ears may be formed. This thesis is exactly in accord with the practical experience that B (Yeoman) and other new high yielding hybrids can exhibit their intrinsic yield superiority only under high farming. Under average or low conditions

A (Squarehead's Master) and the other older wheats are equal in yield to Yeoman (though they are definitely inferior in standing during tempestuous weather).

The conjoint variations in time of the two properties ((α) limitation to formation of ear-bearing tillers, and (β) spatial limitation to tillering) may be represented, as below, in the form (α) minus (β), the negative entries implying that (β) comes later than (α) and conversely, and the unit being one week:

	A		*B*		*C*	
	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.
1926–7	+1	+2	—	—	−1	+1
1927–8 (early sowing)	−4	−2	−3	−1	−5	−3
1927–8 (late sowing)	−3	−1	−2	0	−4	−2

It is not possible to offer an explanation of the seasonal differences these values display. That they tend to be in general negative and in order of magnitude, $B < A < C$, may, however, be inferred. Ear size—and especially its gradation among the ears of a plant—has been shown to be an important attribute or, crudely speaking, varietal yield factor. Further study of critical period incidence (namely (α) and (β) as here used) is therefore clearly desirable. Probably the best form of investigation would be by study throughout crop life of census samples in field crops, since these embrace a wide range of spacings. Seasonal effects will, in turn, call for attention.

V. Varietal development under field conditions and a comparison between spatial response on the plot and in the field.

In the two sets of experiments described in Sections III and IV three wheats (*A* = Squarehead's Master, *B* = Yeoman, and *C* = Rivet) have been compared in connection with yield, and the internal factors of yield. A study has also been made of the responses of these varieties to spacing. From this has emerged evidence upon the general influence of spacing on yield in wheat. The whole of this work was carried out on plots with the seed dibbed by hand at regular intervals and upon a carefully prepared tilth. It was felt desirable to test whether the relative behaviour of varieties, as grown in plots, was maintained under field conditions. To effect this test was the first object of the investigation now to be described. A second object was to compare the results of spacing-plot experiments (Section IV) with the inferences as to spatial response repeatedly drawn from studies of field crops. These inferences

must be briefly summarised. In any field of corn, the plants stand very irregularly in the rows. On one short length of row—*e.g.* a 1-foot length—there may be 5 plants; on the next 23; on the next 17; and so on. The yield of grain from any short length of row has proved to be governed primarily by the number of plants standing upon it. Where there are few plants, the average number of ears per plant and the average ear size are relatively high, and conversely. But response to wider spacing is not marked. As a result, over the general spatial range found in field crops, increased size of plant fails to "compensate" for low number of plants. Thus the rate of yield from a field is largely a reflection of the fluctuating population density.

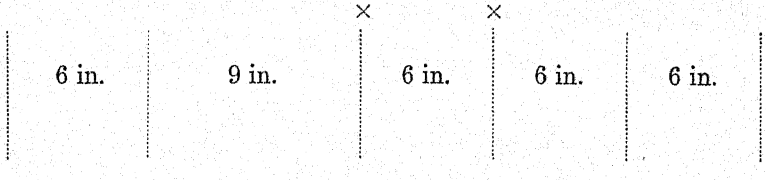
The studies which have brought these facts to light have been in the form of a "census" of an acre. A full account of the procedure may be found in an earlier paper (Engledow(3)). Essentially, it involves periodic counts of plants and tillers on a number of suitably dispersed "samples." Every sample is a 1-foot length of a drill row (*i.e.* row of plants).

Only varieties *A* and *B* were studied, *C* being omitted. It was impossible to work on ordinary field crops because of the statistical requirements proper to an inter-variety comparison. Advantage was therefore taken of the 1927–8 half-drill strip yield trials of varieties *A* and *B* carried out by the National Institute of Agricultural Botany. In this form of trial the two varieties occupy alternate pairs of strips, each half the width of a seed drill, thus:

A B B A A B B A A ....

The trial affords a very accurate measurement of rate of yield per acre while, at the same time, providing the normal drill sowing and the other general conditions of ordinary field crops. A "census of an acre" study of varieties *A* and *B* was made on these strips, 100 samples of each variety being brought under observation (each sample was a 1-foot length of drill row).

In the half-drill strip trial the distance between rows is, according to a special plan, at some points across the strip 6 in. and at others 9 in. The census samples were on rows (×) at the intervals indicated below:



They thus represent a row spacing, partly of 6 in. and partly of 9 in., and may therefore be reasonably compared with the spacing plots described in Section IV in which the rows were 6 in. apart. Sowing dates were for the "field" (*i.e.* half-drill strip trial), November 3, 1927, and for the spacing plots with which comparison is to be made, November 9, 1927. The field crop was on boulder clay in good fertility, the land having been fallow for a year with a crop of mustard ploughed in at the end of August. The spacing plots were on gravel over gault-clay, the land being rather lower in general condition than that which carried the field crop.

All the phenomena commonly observable in census studies of field crops were examined. The passages which follow, deal, however, only with matters strictly relevant to the two main questions which have been propounded.

§ 1. *Comparative varietal development under field conditions.*

In Table XV is presented an outline of development and of the mature populations of varieties *A* and *B* as grown under field conditions and in the 6 in. × 1 in. spacing plots. It will be perceived that the relative behaviour of the varieties under field conditions is precisely similar to what was found in the plot experiments (*vide* Sections III and IV). Thus *B* commenced to tiller before *A* (*cf.* values on February 17) but the proportion of early tillers surviving to form ears at harvest was lower than in *A*. Further, *B* was inferior to *A* in average values of yield per plant, yield per ear, 1000-corn weight and number of grains per ear. The order of difference between the varieties in these characteristics was about the same as in the plot experiments.

The time at which spacing commenced to limit tillering is determinable from the correlation between number of plants per foot and average number of tillers per plant. For *B* this correlation reached a significant value by March 21 ($r = -0{\cdot}40$), at which time it was just not significant for *A*. Thus in the field, as on plots, this phase of the critical period of tillering falls slightly earlier for *B* than for *A*.

It is evident, then, that in the field the internal yield factors or yield attributes of variety *A* show the same behaviour relatively to those of *B*, as on dibbed plots.

§ 2. *Spatial response on the plot and in the field.*

In the field crop the number of plants per foot fluctuated from 0 to 27. This is a far narrower range than most field crops show, the probable reason being the accurate adjustment of the drill and the

general care employed in sowing the half-drill strip trials. For both *A* and *B* there was an average of about 13 to 14 plants per foot of row, and the rows were approximately (as explained above) at 6 in. apart. Thus the field sowing may reasonably be compared with the 6 in. × 1 in. spacing plots sown at about the same time. Table XV offers material for comparison.

Table XV. *A résumé of development and yield for varieties* A *and* B *under field conditions and in the* 6 *in.* × 1 *in. spacing plots.*

		Field conditions		Spacing plots	
	Date	*A*	*B*	*A*	*B*
Sowing	Nov. 3	For field conditions			
	Nov. 9	For spacing plots			
Average no. of plants per foot	Dec. 12	14·0	14·9	—	—
Average no. of side tillers per plant	Feb. 17	0·42	0·75	0·25	0·72
Average no. of side tillers per plant	Mar. 7	1·56	2·17	1·81	2·52
Correlation between no. of plants per foot and no. of tillers per plant	Mar. 7	+0·05	−0·20	—	—
Average no. of side tillers per plant	Mar. 21	2·94	3·65	3·07	3·95
Correlation between no. of plants per foot and no. of tillers per plant	Mar. 21	−0·23	−0·40	—	—
Average no. of plants per foot	Harvest	13·6	14·8	—	—
Average no. of ears per plant	,,	1·21	1·29	1·29	1·45
Percentage of early tillers (Mar. 21) which formed ears	,,	30·7	27·7	31·7	29·3
Average yield per plant (gm.)	,,	1·57	1·37	1·90	1·69
Average yield per ear (gm.)	,,	1·31	1·07	1·45	1·17
Average weight of 1000 corns (gm.)	,,	49·9	43·7	51·9	45·7
Average no. of grains per ear	,,	26·3	24·5	27·9	27·8

There are marked differences for both varieties between development in the field and on the plots. Tillering commenced earlier in the field (*cf.* February 17, Table XV) but speedily became stronger on the plots where it resulted in a greater number of ears per plant at harvest. In all the following attributes both varieties showed markedly higher values on the plots than in the field crop: percentage of March 21 tillers which formed ears; yield per plant; yield per ear; weight of 1000 corns; and number of grains per ear. On the whole, the fertility of the land carrying the field crop was definitely higher than the plot land. It is evident, then, that in the plot experiment there were circumstances more favourable to development and yield than those of the field crop.

It is interesting to determine the chronology of the two properties or phases of the critical period of tillering. Using the notation of Section IV:

(α) Limiting time for the formation of ear-bearing tillers:

6 in. × 1 in. spacing plots: third week in February for *A* and *B* (*vide* Table XIV and Section IV).

Field crop: shortly before February 17 for *A* and *B*.

(β) Time at which spacing begins to limit tillering:
6 in. × 1 in. spacing plots: second week in March for *B*; third week in March for *A* (*vide* Table XIV and Section IV).
Field crop: March 21 for *B* and a little later for *A*.

In the comparison drawn above between the 6 in. × 1 in. spacing plots and the field crop, the latter was treated as a whole. That is to say, average values of population density and tillering were used. Another comparison may now be made. The one hundred samples for each variety may be grouped on the basis of number of plants per foot and appropriate groups compared with the 6 in. × 1 in. and 6 in. × 2 in. spacing plots. A convenient grouping is to divide the frequency distribution of number of plants per foot into five equal frequency parts or quintiles. This is effected in Table XVI in which are shown the yield attributes of the quintile populations. The first quintile, constituted by samples (foot lengths) with from about 1 to 9 plants each, has an average of 6·5 plants per foot. It is thus comparable with the 6 in. × 2 in. spacing plots. Similarly the second quintile with an average of 11·2 plants per foot is comparable with the 6 in. × 1 in. spacing plots. Italicised entries in the columns for these two quintiles show values for the attributes derived from the corresponding spacing plots.

Table XVI. *The attributes of the quintiles of the frequency distribution of number of plants per foot for* A *in the census of the field sowing* (*data in* Roman *type*) *with data for the corresponding spacing plots* (*data in italics*), *namely for* 6 *in.* × 2 *in. spacing for comparison with the first quintile and for* 6 *in.* × 1 *in. spacing for comparison with the second quintile.*

	Quintile				
	1	2	3	4	5
No. of plants per foot (limits of range)	1·0–9·4	9·5–13·1	13·2–15·3	15·4–17·5	17·6–27·0
Average no. of plants per foot	6·5	11·2	13·7	15·9	19·7
Uniform no. of plants per foot	*6·0*	*12·0*	—	—	—
Average no. of ears per plant	1·22	1·21	1·18	1·17	1·17
	2·17	*1·29*	—	—	—
Average yield per plant (gm.)	1·57	1·63	1·59	1·56	1·49
	3·46	*1·90*	—	—	—
Average yield per ear (gm.)	1·28	1·34	1·34	1·34	1·27
	1·59	*1·45*	—	—	—

Close reliance cannot be placed in the actual values of the yield attributes for the quintile populations, since a quintile represents only 20 foot-lengths of row. But, remembering that the field crop was on land in better heart than the spacing plots, certain general inferences

may with safety be drawn from Table XVI. All the yield attributes of the 6 in. × 1 in. spacing plots exceed the corresponding values of quintile number two (11·2 plants per foot) of the field crop. The second comparison—6 in. × 2 in. spacing plot with quintile number one (6·5 plants per foot)—reveals a far stronger superiority of the plot over the field. Actually, average yield per plant on the spacing plot is more than double the value for the comparable portion of the field crop. The great difference must, however, be accepted with caution. In the samples constituting the first quintile are some with 1, 2 or 3 plants only per foot and none with more than about 9. Thus though the average is 6·5 per foot, comparison with the 6 in. × 2 in. spacing plot must not be unduly pressed. It is evident nevertheless, as in the comparison between the field crop as a whole and the 6 in. × 1 in. spacing plots, that development and yield are much more favoured on the plot than in the field.

Finally, a general comparison may be made between spatial response on the plots as opposed to response in the field. For the plots, Table XIII (Section IV) shows that at 6 in. × 2 in. for both *A* and *B* (1927–8, early sown), yield per unit area is of the same order as at 6 in. × 1 in. Reduced number of plants has been "compensated" by greater number of ears and ear size. As between the quintiles of the field crop, however, as Table XVI makes clear, corresponding response to spacing is not found. Thus, as average number of plants per foot rises in the sequence 6·5–11·2–13·7–15·9–19·7 the average number of ears per plant displays the gentle cadence: 1·22–1·21–1·18–1·17. Rates of yield per foot (calculated from the data of Table XVI) are, correspondingly (in gm.): 10·2–18·3–21·8–24·8–29·4. The general inference is thus again clear. Under field conditions plant development shows relatively small response to spacing and number of plants per foot is a very direct determiner of yield per foot.

The disharmony between the plot and the field in yield-spacing relationship is thus very marked. Its causes must now be discussed.

§ 3. *The nature of the difference between field and plot conditions.*

Up to some seventy years ago, much of the corn was "dibbed" in. Holes at regular intervals and in rows 9 in. to 15 in. apart were made by dibbing "tongs" or "irons." The dibber worked with one iron in each hand, and made holes some 6 in. asunder in the row. Sowers followed him, dropping two to four seeds into every hole. Seedings of even two to three pecks are recorded to have given yields often exceeding those to be expected in the same districts from present-day drill

sowings of 2½ bushels per acre. Again, on experimental plots dibbed by hand (as in the plots of Sections III and IV) heavy crops often result from light seedings. In 1925 a barley chessboard of six varieties, 150 plots in all, was sown at the customary 6 in. × 2 in. This is equivalent to a seed rate of just over 1 bushel per acre. The rate of yield for the 150 plots was 55·7 bushels per acre. From an ordinary field crop on the same land and in the same season, 40 bushels would have been considered a very satisfactory yield. Similarly the 6 in. × 1 in. spacing plots gave a yield (calculated from yield per plant and number of plants) at the rate of $A = 33{\cdot}2$ cwt. and $B = 29{\cdot}6$ cwt. per acre (of grain as dry matter). From the half-drill strip trial, on which the census was made, the actual yields were $A = 25{\cdot}4$ cwt. and $B = 25{\cdot}3$ cwt. per acre (of grain as dry matter).

Theoretically, the disharmony of evidence as to spatial response as between the field and the spacing plots, admits of two explanations. It may be suggested that those census samples with very few plants per foot were the ones that happened to be situate on poor "patches" of soil. This would require that the "badness" of such a patch was responsible both for the smallness of the number of plants on it and their poor development. Now careful census studies have shown that the number of plants at harvest on any foot length of row is primarily determined by the number of seeds deposited there by the drill. Critical study suggests, however, that the number of seeds deposited on any short length of row is determined not by the "goodness" or "badness," or any other property of the soil on that length. It is governed entirely by the mechanical action of the drill at the point concerned. From point to point along a row the delivery from every coulter of the drill is highly fluctuating. A full discussion of this matter may be found in an earlier paper (Engledow(6), pp. 28–32).

It appears, then, that some other explanation must be sought for the differences which have been demonstrated between the plot and the field. There remains the possibility that cultural conditions are far more favourable on hand-dibbed plots. This is believed to be the case and the following considerations are urged:

(i) On hand-dibbed plots spacing is regular. In the field even over a 1 ft. length of row, it is highly irregular. By a special method (Engledow(6), pp. 15 *et seq.*) counts were made of the distribution of plants per inch of row. On a foot length with 15 plants there might be 3 on the first inch, none on the next 3 inches, 8 on the next 4 inches and 4 on the remaining 4 inches. The average value of 15 plants on

12 inches masks the crowding of 8 plants on 4 inches and the 3-inch blank actually occurring within the foot. How irregularity of this kind affects yield per unit area cannot readily be demonstrated. That it is less favourable in general than regular spacing appears certain.

(ii) On plots, all seeds are sown at the same depth and at a favourable depth. Deep burying severely inhibits the growth of a significant proportion of the plants in a field crop.

(iii) Clods overlie many seeds in fields of winter corn. On heavy land this is an important consideration. A plant may be many days in growing through or from under a large clod.

(iv) The dibbing iron ensures a *locally* firm seed bed, and covering in with a rake provides a perfect top tilth over the seed. By ordinary methods these amenities are virtually unobtainable in the field.

(v) While the field crop concerned was definitely "clean"—on practical standards for winter corn on heavy land—the spacing plots were kept perfectly weed free.

(vi) The frequent spring hand-hoeing on the spacing plots (for weed extermination) may have conferred those benefits always claimed, though never, perhaps, experimentally demonstrated with satisfactory precision, as results of "inter-cultivation."

It is believed that of these favourable circumstances regularity in distribution of seed ranks first in importance.

Whatever the weight of these considerations, it must be accepted that, *ceteris paribus*, hand-dibbed plots offer conditions more favourable to plant development than those of the field. The difference is made manifest in rate of yield. This reflects, essentially, the failure of plants in field crops to show that response to spacing predicated by spacing plot results.

VI. INTER-PLANT AND INTER-VARIETY COMPETITION.

It is customary in all variety trials to discard plot borders in order that yields may not be disturbed by inter-variety competition or interference. In some cases this is obviously necessary, as when tall and short or early and late varieties are included in the same trial. Here competition or interference is, in some measure, a matter simply of shading. But where the varieties are of similar habit any competition must arise from a struggle for water and plant foods or, as has been held, from a form of toxicity. There have been experiments to test the influence of variety competition upon yield. They do not, however,

offer a strong body of evidence, nor are they sufficiently critical to repay a review. The general question has a manifold importance. Mixed cropping dates from the earliest times. In England the "drage" or "dredge" corn, *e.g.* oats and barley or beans and (black eye) maple peas are still found. Widely different crops, two, three, or even more in number are grown together in many parts of India. "Cotton through yams" and other mixed crops are common in West Africa. The mixing of wheat varieties is a popular practice in East Anglia, especially perhaps in Cambridgeshire. Little Joss with Squarehead's Master is often found in the Fens, while on the heavier land Iron and Yeoman, Iron and Wilhelmina, and even Iron and Rivet may be seen. Increased yield from a mixture of varieties is stoutly alleged by advocates of the practice.

A second point of interest is the possibility of reducing the size of plots for tillering studies. If variety competition did not occur, or were absent in early development, varieties could be grown in very small plots, *e.g.* single rows. Soil heterogeneity would thus be reduced and smaller numbers of plants would give statistically acceptable results. It has been shown that among the plants of the same variety, competition (spacing) does not affect tillering until a critical period, which for winter wheat is at about the third week in March.

Finally, "competition" offers an interesting method of comparing varietal developments.

It was, therefore, decided to grow varieties *A* (Squarehead's Master) and *B* (Yeoman) under conditions of intensive competition. Their dates of maturity are practically identical. Two beds were laid out, each with 100 rows at 6 in. apart and with 24 seed holes in the row. In one, *A* and *B* were sown in alternate rows with two seeds in every hole. In the other, one seed of *A* and one of *B* were sown in every hole (Exp. 4, List of Experiments, p. 273). Sowing was on November 7, 1927, and general comparison may therefore be made with the spacing plots (Section IV) sown on November 9, 1927, and on an adjoining site. Periodic tiller counts were made, but with *A* and *B* in the same hole it was not possible to count the tillers of the two varieties separately. Full harvest data were procurable as the varieties were separable on chaff colour (*A* = red, *B* = white). To strengthen the basis of comparison each bed was harvested in five blocks each of 20 rows. Corresponding (opposite) blocks of the two beds were compared, giving five comparisons, the block containing 240 plants, less casualties. Where a seed failed or a plant died the surviving "single" was excluded from the counts.

In Table XVII are presented the data of development and yield. It will be observed that in both growings the characteristics of *A* and *B* preserve the general relationship recorded in the other experiments (Sections III, IV and V). Thus, in early life, *B* tillered more than *A* and it produced more ears per plant. Similarly, the percentage of surviving tillers was lower in *B*, as were yield per ear and yield per plant. Inter-varietal competition did not, therefore, disturb broad relative varietal development.

Table XVII. *For* (1) *Varieties* A *and* B *in alternate rows* (2 *seeds per hole*), (2) *Varieties* A *and* B *occupying the same holes* (i.e. 1 *seed of* A *and* 1 *seed of* B *in every hole*). *Spacing of holes in both cases* = 6 *in.* × 2 *in. Showing rate of tiller formation and the attributes of the harvest population.*

	Varieties *A* and *B* in alternate rows		Varieties *A* and *B* occupying the same holes	
	A	*B*	*A*	*B*
Average no. of side tillers per plant on Feb. 22	0·40	0·77	(Not counted)	
Average no. of side tillers per plant on Feb. 29	0·83	1·21	1·09	
Average no. of side tillers per plant on Mar. 22	2·53	3·07	2·95	
Average no. of ears per plant at harvest	1·60	1·60	1·80	1·95
Average yield per plant (gm.)	2·57	1·89	3·05	2·39
Average yield per ear (gm.)	1·60	1·19	1·70	1·23

Comparison between the two growings—*A* and *B* in alternate ranks with *A* and *B* in the same holes—may now be effected. On February 29 the average number of tillers per plant for *A* and *B* together was 1·09: for *A* and *B* in alternate rows it was 1·02. Corresponding values for March 22 were 2·95 and 2·80. At maturity, for number of ears per plant, for yield per plant, and yield per ear, both the varieties gave higher values for plants in the same holes than for varieties in alternate rows. Finally the rates of yield per unit area, judged by yield per seed hole were in the ratio—varieties together : varieties in alternate rows = 5·44 : 4·46. At face value the tillering and mature plant data suggest that "intensive competition" has favoured development and yield in both varieties. That is to say, "mixed cropping" has been advantageous. Tests of probability suggest that, at any rate, it has not been disadvantageous. The scale of the experiment was too small to warrant final conclusions on a matter of such importance. It is suggested, however, that too much importance may have been attached to varietal "interference," and that there should be a full investigation of the subject.

Where one seed failed to germinate or one plant in a hole died in early life, the survivor was kept under observation. Comparison between these singles and the normal doubles afforded a special form of spacing test. The data were naturally limited, but they showed a critical period (for commencement of the influence of spacing on tillering) coincident for both *A* and *B* with that found for the adjoining spacing plot experiment.

A special form of spacing experiment was carried out as an ancillary to the main experiments. It was made upon spring barley, sown at 6 in. × 2 in. The object was to ascertain at what phase of growth, and in what manner, inter-plant competition became effective. Removal of competition at fortnightly intervals was the principle employed. This was effected by uprooting two plants out of every three in the row, thus leaving a population at 6 in. × 6 in. The first thinning out was made just after the critical period of tillering. Five beds, each of 240 plants, were employed for each of the periodic treatments or thinnings. The effect on general growth was sharply marked. Thinning out, especially in the earlier treatments, was quickly followed by a recrudescence of tillering, the plants becoming very bushy in habit. But none of the extra tillers so induced, survived. At harvest the average yield per plot and per plant was not significantly greater for any of the thinning treatments than for the unthinned control. The scale of the experiment was not such as to expose small differences in yield. It showed, nevertheless, that inter-plant competition at a spacing of 6 in. × 2 in. exercised most of its influence on yield before the critical period of tiller formation. Repetition of this form of spacing experiment on a larger scale and with fuller observations on foliar, tiller and ear development, appears likely to further understanding of the nature of inter-plant competition.

VII. The germination and plant survival of wheat.

The germination of seed and the survival of plants cannot be said to have had adequate attention in the consideration of yield-governing factors. In Great Britain most of the cereal samples submitted for laboratory test show a very high (88–98 per cent.) germination; it is usually assumed that germinations of this order may be expected in field sowings. As to plant survival in field crops there has been, until recently, very little evidence. Jethro Tull, from inference rather than test, stated that of about ten seeds of winter corn sown, one might be expected to give a plant at harvest. His opinion has been kept alive

by a sequence of plagiarisms. The considerable body of evidence available from the experiments here under review suggests that current views on both germination and survival of wheat should be revised. And further, they point to these factors as of considerable importance in the yield of field crops. In this connection it is to be remembered that in practice considerable risk is not infrequently run by sowing seed of imperfect maturity, or which has been inadequately screened, or which carries disease. A survey of this matter may be found in reference (2).

It is desirable to deal with three aspects of survival, viz. germination and survival of young plants; survival of the young plant to maturity; and the proportion of seeds sown which give mature plants.

In Exps. 1, 3 *a–d* and 4, carefully selected seed was dressed with formalin and sown at uniform depth and spacing upon a specially prepared tilth. As the results of all the experiments were in general agreement, it will suffice to present, in Table XVIII, those from the four-year variety comparison (Exp. 1). The date of sowing was November 11 in 1926–7 and November 5 in the other three years. A period of two or three months from sowing appears, in general, to be critical for germination, and early survival of winter wheat. It will be observed that in the first three years of the experiment 80–90 per cent. of the seeds gave living plants at two months from sowing. In 1927–8 a Christmas blizzard caused heavy mortality in most of the winter corn, and its results are patent in the table.

Table XVIII. *Germination and survival rates in Exp.* 1 (*variety comparison*).

Survival data	Year	Variety *A*	*B*	*C*
Percentage of seeds sown which survived as plants two months after sowing	1924–5	84	81	79
	1925–6	87	87	86
	1926–7	92	91	91
	1927–8	66	71	50
Percentage of plants alive two months after sowing which became ear-bearing plants at harvest	1924–5	85	83	81
	1925–6	78	66	52
	1926–7	98	97	98
	1927–8	88	94	92
Percentage of seeds sown which grew into ear-bearing plants at harvest	1924–5	71	67	64
	1925–6	68	58	45
	1926–7	90	88	90
	1927–8	59	67	46

Of the plants alive at two months from sowing, about 80–90 per cent. grew into ear-bearing plants at harvest. Favourable weather led to exceptional survival in 1926–7. The unusually low values of 1925–6 are due entirely to a severe attack of wheat bulb-fly. No less than 88 per

cent. of the population of the plots was attacked and, in the circumstances, the survival rates are remarkably high.

Survival as between seeds sown and harvest plants is very variable. The highest and lowest values occur in those years in which survival at two months was respectively highest and lowest.

The general inference is that in ordinary seasons and in absence of severe parasitic damage, the strength of the harvest population is largely governed by the progress of development and growth in the first two or three months from sowing. This implies that in practice good seed, a suitable tilth, and timely sowing (*vide infra*) exercise an important effect upon yield. In the three aspects of survival which have been discussed, variety *C* (Rivet) tends to be inferior to *A* and *B* (Squarehead's Master and Yeoman).

Evidence for field conditions may be found in the following: Engledow(3); Doughty and Engledow(4); Doughty, Engledow and Sansom(5). In the field, briefly, some 60–80 per cent. of the seeds sown appear to grow into ear-bearing plants at harvest. Most of the losses arise either from failure to germinate, or from the dying out of seedlings in the first two or three months from sowing. Unusually adverse weather or parasites may greatly reduce the survival; but the early months of life must still be regarded as the principal plant-mortality period. The low casualty rate of the period between about two months after sowing and harvest, was strikingly brought out in field studies conducted on a uniform plan in 1928–9 in Hertfordshire (Hertfordshire Institute of Agriculture), Norfolk (Norfolk Agricultural Station), and Cambridge (School of Agriculture, twice repeated—on heavy and on extremely gravelly soil). The percentages of plants which survived from about two months after sowing to harvest were:

Hertfordshire	59	per cent.
Norfolk	96	,,
Cambridge (heavy land)	82	,,
,, (light land)	95	,,

Survival was thus extremely high save in Hertfordshire, where severe wheat bulb-fly attack adequately explained the low rate. These figures are the more remarkable, in that in 1928–9 an altogether exceptionally severe winter was followed by a no less exceptional drought. On the light land Cambridge trial ear-development was severely inhibited but plant survival was extremely high.

In these field experiments and in similar ones at Cambridge in 1927–8

(*vide* Doughty, Engledow and Sansom(5)) one point at issue was the developmental effect of nitrogenous top-dressings, applied at varying periods between sowing and the end of February. It appears certain that the marked influence of such dressings upon yield is unconnected with plant survival rate, which is the same for all treatments and for the untreated control.

In the field, germination is much affected by the physiological condition of the seed, by the seed bed, and by drilling conditions. An experiment specifically to test good and bad seed, and very "rough" seed bed conditions, was carried out over a three-year period. The procedure is given in the List of Experiments on p. 273. A succession of counts was made, and percentage germination in the field represents in all cases the maximum number of plants found. Table XIX contains the results. In connection with "good" and "bad" seed of *B* (Yeoman) there appears a number of interesting features. In general, it is evident, very rough conditions result, for good and bad seed alike, in far lower germination in the field than in the laboratory. In this experiment, the conditions (*vide* List of Experiments, p. 273) were made extremely severe. But present-day wheat growing on heavy land leans markedly towards a bare minimum of cultivations, and loss of potential yield through very low germination undoubtedly occurs in many cases. The data for 1925–6 and 1926–7 emphatically show the great importance of "good" seed under adverse sowing conditions. That the two samples of *B* in 1927–8 show about the same field germination may probably be accepted as a result of unusually severe weather. The periodic counts show that the good seed germinated much more rapidly than the bad, and many of the earliest seedlings were killed off. The seedling population from the bad seed, appearing above ground later, suffered far less from weather. In many reported trials of good *versus* bad seed, little difference in final yield has been found. But, as a rule, the criterion of goodness has been size of seed. This, though of some importance, matters far less than physiological condition. Immaturity or prolonged exposure to wetness at harvest or in the stack are the commonest causes of physiological imperfection, which almost invariably manifests itself in low laboratory germination. But, as Table XIX shows, defects exposed by laboratory test take on a much more serious significance in the field. Briefly, a sample of low laboratory germination contains, besides a proportion of non-germinable grains, a considerable proportion which can germinate and survive in the field only if conditions are very favourable.

Table XIX. *Percentage of seeds which germinated under very rough field conditions* (*Exp.* 5).

		Variety and type of seed			
	Year	*A* (good)	*B* (good)	*B* (bad)	*C* (good)
Laboratory germination (%)	1925–6	—	99	80	98
Field germination (%)		—	65	32	31
Laboratory germination (%)	1926–7	99	99	74	98
Field germination (%)		42	37	17	34
Laboratory germination (%)	1927–8	98	98	75	96
Field germination (%)		18	17	14	17

Leaving out of account the exceptional year 1927–8, it may be inferred from Table XIX that *C* (Rivet) tends to germinate less successfully than the other two varieties. The data of the hand-dibbed plots of Exp. 1 (*vide* Table XVIII) also support this inference. Through the kindness of the Chief Officer of the Official Seed Testing Station it is possible to adduce evidence from commercial seed. Samples are first counted for germination on the fifth day, and the percentages given below are fifth-day values based upon 100 tests per variety. Varietal comparison is limited to practicably "good" seed by the exclusion of all samples which failed to give 90 per cent. germination or more at the second count, *i.e.* tenth day:

Variety	% germination (at fifth day)
(*B*) Yeoman	88·3
(*A*) Squarehead's Master	81·8
(*C*) Rivet	70·2

The inherently lower germination capacity of Rivet wheat is thus again evident.

In both laboratory and field, low germination and slowness of germination are characteristically associated. Both are, indeed, manifestations of physiological imperfection. It is to be anticipated, therefore, that variety *C* (Rivet) would be tardy in germination as compared with *A* and *B* (Squarehead's Master and Yeoman). Proof of this arises clearly in Exps. 1, 3 *a–d* and 5, and the data of Exp. 3 *d* (variety spacing experiment, 1927–8, sown January 25, 1928) are given in Table XX as an illustration. As between close and wide spacings the differences are small save for *C* (Rivet). The order of speed of germination, viz. $B > A > C$, agrees with that of the Official Seed Testing Station data for germination capacity of commercial samples. Moreover, in the remaining dibbed-plot experiments, the same order is maintained, although inter-varietal differences are in some cases slight. Thus rate of germina-

tion appears to be a varietal character, which in Rivet, and possibly in some other varieties, has significance in relation to yielding capacity.

Table XX. *Rates of germination of varieties* A, B *and* C *sown on Jan.* 25, 1928 (*the values show the percentages of seeds which had germinated at each of three counts*).

	Percentage of seeds germinated					
Variety	*A*		*B*		*C*	
Spacing	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.	6 in. × 1 in.	6 in. × 2 in.
Date of count						
Feb. 28	40	41	58	56	38	29
Mar. 1	46	48	61	60	43	33
Mar. 3	59	54	68	67	46	36

Table XXI. *The development of "normal" and "late" germinating plants in Exp.* 1 (*varietal comparison on dibbed plots*), 1924–5.

	A		*B*		*C*	
	Normal	Late	Normal	Late	Normal	Late
Average no. of side tillers per plant:						
Jan. 22	0·71	0·05	0·78	0·14	0·54	0·09
Feb. 19	2·44	0·60	2·59	1·00	2·08	0·80
Mar. 5	3·46	1·06	3·70	2·03	3·17	1·63
Average yield (gm.) per plant at harvest	4·23	2·01	3·49	2·40	5·01	3·72

In the soil, even with hand-dibbed beds, a certain proportion of seed is always very late and germinations up to fourteen weeks after sowing were observed. It is in practice with oats that late and irregular germination is most serious, particularly perhaps with the variety Black Winter. In Exp. 1 (comparison of three wheat varieties in dibbed plots) a separate record was kept of the development of every plant. It is thus possible to compare the life histories of normal and late germinating individuals. The season 1924–5—an "average" season and with no serious parasitic disturbance—may be used as an example. Sowing was on November 5 and germination counts were made from November 27 to December 5. Plants which had germinated by December 2, numbering about 80 per cent. of the seeds sown in all three varieties, were classed as "normal," all which germinated subsequently being grouped as "late." In Table XXI are extracts from the developmental records and the difference between normal and late plants, persisting up to yield at harvest, is very striking. Broadly speaking 20 per cent. of the plants of every variety were "late" and their rate

of yield was some 65 per cent. of that of normal plants. Thus the loss in potential yield per unit area attributable to late germination is of the order of 7 per cent. If such be the result with selected seed carefully sown on an extremely good tilth, losses of 15–20 per cent. may reasonably be surmised to occur in field crops. The association between late germination and poor development is probably, in large measure, a dual manifestation of some physiological defect in the seed.

In Section X plant developmental history is separately traced out for each one of the eight dibbed plots of the three varieties *A*, *B* and *C* used in Exp. 1. There are shown to be considerable ups and downs in "bed history." That is to say, the bed of a variety showing most forward development at one stage of plant growth may, a little later, be by no means foremost. It is interesting to note that, even in respect of germination, bed to bed differences are quite marked. Thus, for variety *A*, the percentage of seeds germinating was:

Bed	I	II	III	IV	V	VI	VII	VIII
% germination on Nov. 27	59·1	67·6	72·4	57·3	78·7	74·2	74·2	57·3
% germination on Dec. 2	83·1	88·0	86·7	77·8	90·2	87·1	87·6	75·6

For carefully selected seeds sown under very favourable conditions, the inter-bed fluctuations in germination are at both counts surprisingly high. Moreover, order of success in germination as among the eight beds displays changes in the brief period of five days between the two counts. These facts reinforce what has already been adduced as to the sensitiveness of the germination process to external conditions. In field tilths all the factors of soil heterogeneity are far more emphatic than in experimental plots, so that the importance of good seed and good tilth in relation to plant development is manifest.

The influence of weather upon plant development is reviewed in Section X. Here may be noticed the effect upon germination. In Table XXII are shown for variety *A*, Exp. 1, the numbers of days from sowing in which germination became complete (excluding the small proportion of very late cases); the total rainfall in the period of four weeks from sowing; and the accumulated temperature for the period of four weeks from sowing (accumulated temperature for a period is taken as the sum of the daily differences between 32° F. and mean daily temperature for all the days of the period). It is inferred that the rapid germination of 1924–5 reflects favourable rainfall and temperature. In 1926–7 rainfall was little more than half of the amount of 1924–5, but accumulated temperature was relatively high. Again then, favourable conditions, particularly of temperature, may be held to account for

swift germination (27 days for completion). The somewhat low temperatures of 1927–8, despite a rainfall greater than in 1926–7, extended the completion of germination to 35 days. Actually, in the fifth week after sowing in 1927–8, the accumulated temperature rapidly attained 300° F. and it was in this week that rate of germination showed marked acceleration. The outstanding year is 1925–6, in which both rainfall and temperature were adverse during the first four weeks after sowing. Accumulated temperature did not reach 300° F. until towards the end of December—about seven weeks after sowing—and it was at this time that germination rate suddenly became rapid. The influence of both rainfall and temperature upon germination thus stands out clearly, and it appears that temperature is likely to be the more important. It is for this reason that unusually early sowings of winter corn lead as a rule to better results than unusually late ones. In many districts of the Eastern Counties, there is an accepted day by which it is considered desirable that all the winter wheat should be "in." These days, for most districts, centre round about the 25th day of November.

Table XXII. *Rainfall and accumulated temperature during the first four weeks after sowing and the number of days in which germination was completed; data for variety* A *in Exp.* 1 (*varietal comparison of three wheats in hand-dibbed plots*).

Year	No. of days (from sowing) in which germination was complete	Rainfall (inches) in first 4 weeks after sowing	Accumulated temperature (° F.) in first 4 weeks after sowing
1924–5	22	2·38	344·5
1925–6	57	0·84	109·5
1926–7	27	1·34	283·5
1927–8	35	1·66	250·5

The evidence upon germination and plant survival of wheat, arising from this series of plot and field experiments, may now be summarised. Under reasonably good conditions seed germination is about 80–90 per cent. The first two or three months after sowing are the principal casualty period, and of the plants which pass successfully through it some 80–90 per cent. survive to harvest. Thus of the seeds sown 60–80 per cent. may be expected to grow into harvest plants. Parasitic action, adverse weather, bad tilth, and bad seed (*i.e.* physiologically imperfect) may bring about very substantial reduction in these rates. Rivet wheat, as compared with Squarehead's Master and Yeoman, is inherently poor and slow in germination. In all varieties tardy germination is reflected by backwardness at all stages of development and by low plant yield.

Rainfall, and still more temperature, profoundly affect rate of germination, and the weather of the first four weeks after sowing is of critical importance. The whole body of evidence clearly points to the practical importance of good seed, suitable tilth, and early sowing.

VIII. A CONSPECTUS OF THE DEVELOPMENTAL HISTORY OF THE WHEAT PLANT.

At present the internal factors of yield are expressible only in terms of morphology. Detailed study of morphological development and its chronology is thus essential in yield analysis. It is also highly important in interpreting the nature of parasitic damage, of fertiliser action, and of weather influences. Surprisingly little attention has been given to it. Certain phases of development in cereal plants have been closely studied, *e.g.* grain maturation. But of the course of development throughout life there are virtually no records. Kreusler and his colleagues many years ago (*Landwirtschaftliche Jahrbücher*, 1877–9) traced out in great detail the development of maize. While they recorded certain morphological stages, such as date of flower formation, their data largely bear upon growth in terms of leaf area and dry weight. The conspectus to be given here has been compiled from the experiments described in the sections above, and from the plots and fields upon which studies have been recorded in earlier papers of this series. An important addition has recently been made by the work of Mr P. S. Hudson (to be published shortly) on the details of development of the wheat ear. It was carried out on Yeoman wheat from the plots of Exp. 1 (*vide* Section III).

The life-history of the wheat plant may be divided, arbitrarily but conveniently, into the following main periods: germination and early survival; early leaf formation; early tillering; late tillering and tiller mortality; ear development and flowering; grain formation. In describing these periods consideration is limited to typical English winter wheats (*e.g.* Squarehead's Master and Yeoman) growing in the Eastern Counties. It must be understood that the suggested chronology of events, while representative, cannot be expected to apply closely to any one specific case.

The major observable events of morphological history are tiller formation and ear formation. In the last-named are two happenings, namely, differentiation of primordial spikelets, and formation of florets in the spikelet. There is a striking feature common to all these "formation" processes. The organs invariably form in excess; commencing

with the latest formed of the series they cease development and die off in succession; and finally less than half survive to maturity. In this characteristic behaviour there probably lie important possibilities of increasing the yield of field crops. Any controllable circumstance such as varietal features or fertiliser action by which initial formation and subsequent mortality of organs are affected, offers scope to plant breeding and crop husbandry improvements. It is only by ascertaining the detailed course and chronology of development, however, that such possibilities can be revealed.

In a general outline, description must be based upon the "average" plant. Development of the individual plant in any set of circumstances, however carefully controlled, is astonishingly variable. For instance, in the spaced and carefully managed plots of Exp. 1 the mature population was widely diversified (cf. *e.g.* Table V). As part of an earlier investigation (Engledow and Wadham (7), Part II, pp. 76 *et seq.* and Table XXXI, p. 94) the mature plant population of a spaced bed was successively selected until there remained only plants which had grown under strictly the same conditions, and in which major morphological development had been similar. The yields of grain per plant in this exclusive population fluctuated from about 300 to 500 centigrammes. In field crops the hazards are even greater than on the experimental plot.

Leaf formation and death follows a course somewhat similar to that of tiller or ear formation, but in which arrest of development and dying off are slower. No records are available. In studies on the influence of rust attack at different periods and in other branches of enquiry, data upon the effective (still active) as opposed to the total leaf area would be valuable. The first green leaf of the graminaceous plant is characteristically different in shape from the rest and commences to die, from the tip, within a few weeks of germination. Somewhat rapid loss of chlorophyll occurs in all but the top two to three leaves of the stem in June.

Of the course of root development extremely little is known. Some observations have been recorded by Weaver *et al.* (14) for wheat in the United States.

Germination and survival.

Winter wheat is sown from the end of September to the end of December. From mid-October to mid-November is usually considered the most desirable time. Germination in plots takes place in $2\frac{1}{2}$ to $3\frac{1}{2}$ weeks and in the field in 3 to 5 weeks. Heavy frosts not infrequently prolong this period to 7 or 8 weeks. A germination percentage of 80

may be expected under reasonably good field conditions. Very wet or immature seed, deep burying of seed, or a very wet and cloddy tilth, sometimes reduce germination to 50 per cent. Rainfall and, still more, the accumulated temperature in the first month after sowing, exercise a marked influence on rate of germination. A certain proportion of grains is always tardy in germination, occupying up to 14 weeks in the process. In general, late germinating seeds develop badly, the resulting plants being of inferior growth and low in yield. The first three months after germination is the chief period of plant mortality. Of the plants which safely pass through this period, some 80–90 per cent. survive and give ears at harvest. The customary spring top-dressing of nitrogenous fertiliser appears not to affect survival rate of plants. In typical field crops the average number of plants per foot at three months after germination ranges from about 8 to 18. If the average be less than 13 to 16 there is usually a loss in potential yield. Within any one field the number of plants fluctuates very sharply and irregularly from point to point along the rows. Typical limits of this fluctuation are 0 to 40 plants per foot. It is traceable, not to tilth nor to casualties, but primarily to irregular deposition of seed by the drill.

Early leaf formation.

Rate of leaf formation varies greatly with season. Sowing in early November, with germination a month later, may be expected to give plants in which the second leaf has definitely begun to emerge by the middle of January, and the third by the beginning of February. Much earlier development sometimes occurs. As among the plants of a population late leaf formation follows late germination and, in turn, presages relatively low and late tillering. There is a fairly general tendency for the fourth leaf of the main axis to appear at about the same time as the first side tiller.

Early tillering.

Side tillers begin to appear some 10 to 15 weeks after sowing. In very cold seasons they may be much later. They appear in a time sequence which reflects variety, soil and season. Denoting the main axis by T_0 and the successive side tillers by T_1, T_2, ... the following may be suggested as representative data:

On experimental plots: T_1 appears 60 to 70 days after germination; T_2 appears 13 to 16 days after T_1; T_3 appears 15 to 17 days after T_2; T_4 appears 12 to 15 days after T_3.

On field crops (average conditions): T_1 appears 80 to 90 days after germination; T_2 appears 20 to 30 days after T_1; T_3 appears 20 to 30 days after T_2.

On field crops (bad conditions): T_1 appears 90 to 100 days after germination; T_2 appears 25 to 35 days after T_1; T_3 appears 25 to 35 days after T_2.

Tillering appears to show phases, or periods, characterised by rate of formation. Existing data is not, however, such as to justify general inferences as to their chronology or significance.

In field crops, where population density fluctuates sharply from point to point along the row, the expected negative correlation between density and number of tillers per plant commonly attains a significant value at about the end of March. Up to this time, therefore, spacing or population density exercises no influence on tillering. It is, as a broad rule, only on the tillers which appear by the end of March, that mature ears are formed. Thus this time, for two reasons, may be regarded as the "critical period" of tillering.

Wheat bulb-fly (*Hylemyia coarctata*) attacks are not uncommon on wheat in the Eastern Counties. They are most severe on crops taken after a fallow. Some 50–80 per cent. of the plants may be attacked and ploughing up often becomes necessary. Early February is perhaps the general time at which evidence of attack is first seen. A timely application of nitrogenous fertiliser may do much to lessen the effect on final yield by inducing the formation of fresh tillers before the critical period.

The strong predetermining influence of early circumstances is clearly reflected in the comparative development of individual plants. In Exp. 1 (Section III) week to week changes were noted for plants with 0, 1, 2, etc., side tillers respectively. On February 5 for one variety 13 per cent. of the plants had no side tiller. A week later 66 per cent. of this subpopulation still remained without side tillers, and 31 per cent. had formed a single side tiller. There were, on February 5, 41·5 per cent. of plants with one side tiller. In the ensuing week 73 per cent. of these produced a second side tiller. Correspondingly of the plants with two side tillers 58 per cent. added one or more side tillers each. Thus the plants with no side tillers on February 5 showed less tendency to increase in tillering than the more advanced ones. A corresponding check for the period February 12 to February 19, again showed the relatively

slow growth of the backward plants. That plants backward in early tillering definitely tend to be later than and inferior to others as maturity is approached, was shown in an earlier study (Engledow and Wadham (7), Part II, pp. 72–3).

Late tillering and tiller mortality.

Tillering continues, in general, throughout April. It may linger into May or even later, but the last-formed tillers frequently display no more than one leaf and this rarely exceeds an inch in length. A very wet season may induce a recrudescence of tillering in July or August, and badly laid crops (particularly in barley) often show a troublesome "second growth." Of the side tillers present at the end of April some 50 to 80 per cent. die without protruding an ear. The latest formed are the first to die and dying tillers may usually be seen by the end of May. The survival rate of side tillers decreases as population density increases within the density limits of field crops. Rainfall shortage in May and June appears to exercise a great influence on tiller survival[1]. One of the most important problems of wheat growing in England is to devise means of increasing the survival of tillers to the ear-bearing stage. Evidence at present available suggests that this is not to be done by the customary spring top-dressing. Late tillers, *i.e.* those which die without protruding an ear must, upon existing knowledge, be regarded as deleterious. They are, in a sense, parasitic upon the earlier, fruitful, tillers; and the analogy between the unpruned apple tree and the graminaceous plant is of considerable speculative interest.

A superabundance of late tillers and foliage, followed by disappointing scantiness in ear formation, is sometimes observed in very early sowings, or where nitrogen has been applied in excess. Yellow rust (*Puccinia glumarum*) and mildew (*Erysiphe graminis*) may infest wheat at any time from mid-December onwards, but May is probably the commonest period of severe attack. The versatility and wide time incidence of *Fusarium* (*culmorum* and *avenae*) have been clearly established by Bennett (15).

In April and early May wheat crops often show a marked yellowing of foliage, sometimes referred to as "May-sickness." Bennett has shown that in some cases this arises from *Fusarium* attack. In the Eastern Counties it appears frequently to be traceable to waterlogged soil and the spring dressing of nitrogenous fertiliser has proved a valuable remedy.

[1] For the general influence of drought see Appendix II.

Ear development and flowering.

Chronologically, ear development is merged with tillering, but it is convenient to discuss the processes separately. In typical winter wheat the growing point of the main axis of the plant begins to be observably differentiated into a primordial ear between the third week in March and the middle of April. In the side tillers differentiation follows at intervals of a few days. By mid-May all save the very small tillers show ear primordia. Death of a side tiller is presaged by cessation of ear development. This occurs first in the latest-formed tiller of the plant in which it may be apparent within one or two days of the commencement of ear differentiation. The spikelets of the ear and the florets of the spikelet also develop and die off in sequence. Hudson's observations show that floret formation in a spikelet may go on from the beginning to the end of May. As many as nine florets may form. The ninth ceases to develop on the day of formation, the eighth follows, and so in sequence. Four florets may attain maturity, of which three may become fertilised and set a grain.

In the typical cases to which this conspectus refers ear emergence takes place on about June 20. Very late but practicably possible sowings may defer it by 7 to 10 days and the severe frost and drought of 1928–9 induced delay of this order. An interval of 80 days may separate the appearances above ground of the main axis and the latest surviving tiller of a single plant. But, as a rule, the whole of the ears of a large population emerge within a period of about 20 days. Size of ear, both in number of grains and average grain weight, decreases in order of time of formation of the stem. Moreover, average ear size increases with number of ears per plant. Typical values for a field crop may be (in gm. and for a water content of 15 per cent. and where E_0 = main axis ear, etc.):

Ear	1-ear plants	2-ear plants	3-ear plants	4-ear plants
E_0	1·12	1·39	1·47	1·62
E_1	—	1·02	1·21	1·36
E_2	—	—	0·98	1·12
E_3	—	—	—	0·84
Average	1·12	1·21	1·22	1·24

The average number of ears per plant varies widely with environment. Specially grown plants have produced a hundred or more ears. In field crops the general limits are 1·0 to 2·0, and a crop of 50 bushels per acre may have an average of 1·8 ears per plant and 23 grains per ear. There is a negative correlation between population density on any small

length of row and average number of ears per plant. Values for typical field crops have usually been of the order $r = -0{\cdot}5$.

Eggs of the wheat midges (*Cecidomyia tritici* and *Contrarinia tritici*) may be seen in the florets within a few days of ear emergence. Very early or very late flowering varieties appear to suffer little from this pest, which in some years (*vide* Appendix II) may reduce the yield by as much as 25 per cent.

Grain formation.

For crops of the kind here considered harvest maturity would be reached at about August 10–17. There have been many studies of grain filling and maturation. Most have been chemical but, dispersed among a number of papers, a certain amount of morphological and cytological evidence is to be found. This has been reviewed in an earlier paper (Woodman and Engledow (12)) and brought into time relation to the stages of chemical development. From the point of view of yield analysis, the most important result of chemical study is the division of grain formation into three periods. In the first the amount of water in the grain steadily rises; it is almost constant in the second; and in the third rapid desiccation occurs. Variety and other circumstances control these periods which have been shown in two separate studies on widely different varieties to be (in days):

	1st period	2nd period	3rd period
Brenchley and Hall (13)	22	18	6
Woodman and Engledow (12)	40	10	15

The actual dry weight of the grain increases until about 8 to 10 days before maturity and then shows slight decrease. Ripening, the final process of grain formation, is essentially a desiccation. No new material enters the grain nor is there any marked chemical change. To complete this conspectus an outline of results from an earlier experiment (Woodman and Engledow (12)) may be given. Red Fife wheat was used, so that for a typical English winter wheat all the periods would have to be slightly lengthened. Using time of emergence of ears as zero day, the chief stages of grain formation occur as follows:

Day 10–12 = first division of the fertilised ovum.

Day 13 = formation of endosperm cells actively in process.

Day 21 = aleurone layer partly formed; infiltration of starch into endosperm cells commencing.

Day 31 = upper endosperm cells full of starch; first leaf of embryo differentiated.

Day 36–7 = first pair of primary rootlets of embryo observable.

Day 40 = end of period of increase in amount of water in grain; grain $\frac{3}{4}$ full length, milky-cheesy in consistency, and definitely green in external colour.

Day 47 = grain at maximum length (decrease occurs from now on); amount of ash in grain ceases to rise; amount of non-protein nitrogen begins to decrease (by conversion).

Day 50 = end of period of constancy in amount of water and commencement of desiccation; external green colour of grain definitely tinged with yellow-brown; infiltration of starch into endosperm cells practically complete; increase of total nitrogen in grain, hitherto rapid, becomes slow.

Day 54 = disintegration of nuclei of endosperm cells commences (induced by the pressure of starch infiltration).

Day 65 = ripening complete (actually a field crop would be cut 7–10 or more days earlier than this).

In crops heavily attacked by yellow rust grain filling is always imperfect. At what periods in life-history the fungus most severely disturbs plant metabolism is not known, nor is it possible to measure the consequent loss in yield. To most English farmers rust is a familiar "blight" which, since it does not kill the plants, is of no great moment. From the characteristic imperfections of grain associated with rust attack, there can however be no doubt that even in England rust is of considerable importance in limiting yield per acre.

IX. WEATHER AND PLANT DEVELOPMENT[1].

In attempting to measure the influence of weather upon crops there are three fundamental requirements. Observations must be continued over a great number of years: this is evident from the various well-known statistical studies upon weather and yield. Again, crop data must be developmental. While valuable advances have come from correlation studies in which the only crop data have been yield, fundamental progress must lie in relating plant growth to the weather influences by which its stages are governed. Finally, what may be called indirect weather effects must be the subject of separate investigation. Thus a very wet autumn may involve late drilling or, if sowing be at the normal time in such a season, the seed bed may be exceptionally rough. Similarly, violent storms in late July may cause extensive lodging and therefore, in field crops, considerable loss of yield (*i.e.* corn actually harvested). In these examples rainfall may be said to have influenced plant growth directly (by water supply, etc.) and indirectly. While no weather factor has a simple influence on growth it is important to exclude indirect influences as far as possible.

Some of the experiments described in foregoing sections partially satisfy the three requirements. This is especially the case with Exp. 1

[1] For the influences of drought see Appendix II.

(Section III) in which three wheats were sown on the same date, on the same experimental field, and with the same careful hand methods, every year. The experiment was carried on for four seasons only. As, however, so little critical crop-weather data are anywhere available, a brief examination of the four-year results is here made.

The impressive magnitude of year-to-year differences has repeatedly been noticed in the foregoing sections. In relation to yield analysis one of the most important effects is seasonal variation in the two phases of the critical period of tillering. This has been fully described in Section IV, § 4, but it is not possible to offer an explanation in terms of weather factors. The influence of rainfall and temperature upon germination has been discussed in Section VII. In following, side by side, the development of the plant and the phases of the weather in the four years, many interesting associations suggest themselves. Inadequacy of data, inevitable in results covering only four years, makes it impossible to press these associations. It is proposed, therefore, to illustrate weather effects simply by an examination of tillering in two stages, before and after the critical period of tillering. The only weather data brought under review are rainfall and accumulated temperature (the accumulated temperature for any period is taken as the sum of the differences between mean daily temperature and 32° F. for all the days of that period).

The time at which tillering "starts" cannot be satisfactorily defined. By extrapolating from the time-tillering curves it is possible, however, to select the date by which some 10 per cent. or so of the plants had one side tiller each, *i.e.* by which tillering had definitely started. These dates were (where Jan. II = second week of January, etc.): 1924–5 = Jan. II; 1925–6 = Feb. II; 1926–7 = Feb. I; 1927–8 = Feb. II. Now for all the years except 1926–7 the start of tillering coincides fairly closely with the attainment of a value of 500° F. in the accumulated temperature, measured from date of germination. In 1926–7 tillering started when accumulated temperature had reached only 400° F. This season stands out from the other three by reason of the low rainfall between germination and the start of tillering. Autumn wetness appears very commonly to retard growth of wheat. It is, therefore, possible that relatively dry soil in 1926–7 induced the onset of tillering in spite of the relatively low accumulated temperature.

The first stage of tillering, *i.e.* roughly, up to mid-March, may now be examined. In Table XXIII are given weekly values of rainfall, accumulated temperature, and tillering increase (as defined in the legend above the table). Taking the four years as a whole, rapid tiller increase

seems to show association with relatively high temperature. The year 1924–5 is noteworthy for its low average temperatures and relatively slow increases in tillering.

Table XXIII. *Showing for the four seasons* 1924–8, *for variety* A, *the weekly increases in tillering; also rainfall and accumulated temperature for every week in the period Jan.* 22 *to March* 11 [*increase in tillering* = 100 × (*increase in average number of side tillers per plant in any one week*)/ (*average number of side tillers per plant at the end of the previous week*)].

	1924–5			1925–6			1926–7			1927–8		
Week ending	Rain (in.)	Accum. temp. (° F.)	Increase in tillering	Rain (in.)	Accum. temp. (° F.)	Increase in tillering	Rain (in.)	Accum. temp. (° F.)	Increase in tillering	Rain (in.)	Accum. temp. (° F.)	Increase in tillering
Jan. 29	0·05	51	—	—	—	—	—	—	—	—	—	—
Feb. 4	0·50	79	31	—	—	—	0·11	53	—	—	—	—
Feb. 11	0·39	87	49	—	—	—	0·36	27	13	—	—	—
Feb. 18	0·74	57	17	1·69	66	—	—	53	29	0·27	97	—
Feb. 25	0·92	28	15	0·26	116	150	1·67	81	116	—	63	148
Mar. 4	0·33	64	25	0·21	103	44	0·83	103	58	0·09	94	67
Mar. 11	0·12	54	21	0·04	106	40	0·22	71	37	0·11	48	38

From mid-March to the end of April may conveniently be taken as the second period of tillering. Weekly data show results generally resembling those for the first period. It is of interest to compare the years in pairs, and Table XXIV contains relevant data. In the seasons 1924–5 and 1926–7 tillering was relatively advanced by mid-March, the respective values of average number of tillers per plant for variety *A* being 4·19 and 2·73. Rainfall was heavier in 1926–7 (2·29 in.) than in 1924–5 (1·78 in.) but the distribution was similar in the two years. Increase in tillering in the period was, however, widely different (Table XXIV), and it is noteworthy that the year of higher accumulated temperature showed almost double the tillering increase of the other year.

The seasons 1925–6 and 1927–8 may similarly be compared. In both, tillering was relatively low by mid-March (respective averages of 1·27 and 1·66 side tillers per plant). Rainfall was not very different for the two years. Now in 1927–8 tiller increase was of almost double the value attained in 1925–6. Here accumulated temperature offers no explanation, for 1925–6 was, in the period under discussion, decidedly the warmer. Distribution of rainfall may have been responsible for the effect. In 1927–8 rain fell in every week of the period; distribution was in fact ideal. But in 1926–7, of the six weeks constituting the period, three were entirely rainless.

Table XXIV. *Showing for the four seasons* 1924–8, *in the period March* 12 *to April* 22 *the total rainfall and total accumulated temperature and for variety* A *the total increase in tillering* (*defined as in legend to Table XXIII*).

	1924–5	1925–6	1926–7	1927–8
Rainfall (inches)	1·78	1·70	2·29	1·50
Accumulated temperature (° F.)	499	705	619	442
Percentage increase in tillering	57·5	167·7	109·9	312·0

Perhaps the most remarkable "seasonal" difference in the whole of the experiments described in foregoing sections is that of the year 1927–8 in the spacing experiments (Section IV). The three wheats were sown on two dates, November 9 and January 25. Much better growth and heavier yield resulted from the later sowing. This is particularly remarkable for variety *C* (Rivet), which is a very late maturing wheat. The early sowing was subject to harsh conditions almost from germination, and was severely checked by the severe frost and blizzard with which the year 1927 closed. In contrast, the late sowing experienced favourable weather throughout early life. Its ear-bearing side tillers, while chronologically late, developed with great rapidity under conditions highly favourable to development of the ear primordia.

X. Soil heterogeneity as reflected by plant development.

The universality of soil heterogeneity has caused the soil to be regarded as marked in some cases by "patches," in others by "fertility gradients." Inequalities of soil composition, texture, drainage, and treatment, of parasitism or weeds, and of many other factors, produce the irregularities in crop areas ascribed, in dealing with yield results, to soil heterogeneity. Little has been done to probe the causes. A first essential step is to map out the patches, that is, the space distribution of heterogeneity. A number of criteria of heterogeneity may be employed. Haines and Keen (16) have made valuable use of the draw-bar pull of the plough in soil mapping. The biological importance of this index is apparent from the association between draw-bar pull and growth which Eden and Maskell (17) have demonstrated. Progress of plant growth, in the form of periodic observations on development, is another important criterion of soil heterogeneity. It provides, during the growing season, a succession of mappings. Moreover, it may help to analyse soil heterogeneity, in contrast with simple data of final yield which integrate the component factors of heterogeneity.

It is proposed to utilise the data of Exp. 1 (Section III) for mapping soil heterogeneity. Here, as in any method of mapping, it is necessary to recognise certain inevitable limitations. In mapping any area of soil, whatever the criterion of heterogeneity, results depend upon the size of the unit of observation. In an earlier paper (Doughty, Engledow and Sansom(5), p. 488) an illustration of this principle was given. It was suggested that the soil might be regarded as consisting of "patches" within which were small sub-patches or "streaks." If sample size were of the order of the streaks, observations on adjacency correlation and other features might be considerably different from those arising when the sample was of much greater size. In the experiment from which data are here to be employed, the unit for estimation of heterogeneity is a plot 6 ft. × 4 ft. Progress of growth is measured by average number of side tillers per plant. Observations were made only once a week and, since all visible tillers whatever their size are counted, fairly large errors of sampling must be expected.

The general space-distribution of soil heterogeneity on nine dates from January 22 to April 22 for the season 1924–5 may first be considered. There were eight plots of each of three varieties of wheat, constituting a symmetrical block of (5 × 5) 25 plots, of which the centre one was not under observation. Diagram IV illustrates the results. The criterion of plant development is average number of side tillers per plant, determined for every individual plot. Every plot which, on this criterion, exceeds in value the mean of all plots of its own variety, is shaded. The identity of the variety occupying a plot is not indicated, as this is not at present relevant. The plots marked by a cross (in the first and last counts) are those which at harvest exceeded the variety mean for all plots in respect of average number of ears per plant. It will be realised that the distinction, above variety average or below variety average, is not of great statistical import. It is, however, convenient for illustration and appropriate to the somewhat limited data.

The area constituted by above-variety-average plots (Diagram IV) appears to show an evolution in form made up of three stages, viz. January 22 to February 4, February 11 to February 25, and March 4 to March 11. For the interval March 11 to April 22 there are no weekly counts, but there is fair similarity between the distributions on these two dates. The most striking feature is the close similarity between distribution of above-variety-average plots at harvest (marked × on the plan for April 22) and their distribution on April 22 (the date of the last tillering count). This similarity may, perhaps, be accepted as sup-

porting the critical period of tillering hypothesis enunciated in earlier sections. No less striking is the comparison of distributions at harvest (ears per plant) and on January 22 (side tillers per plant) illustrated by the plan for January 22. It may almost be said that plots which started well, finished below average. No explanation of this can be suggested.

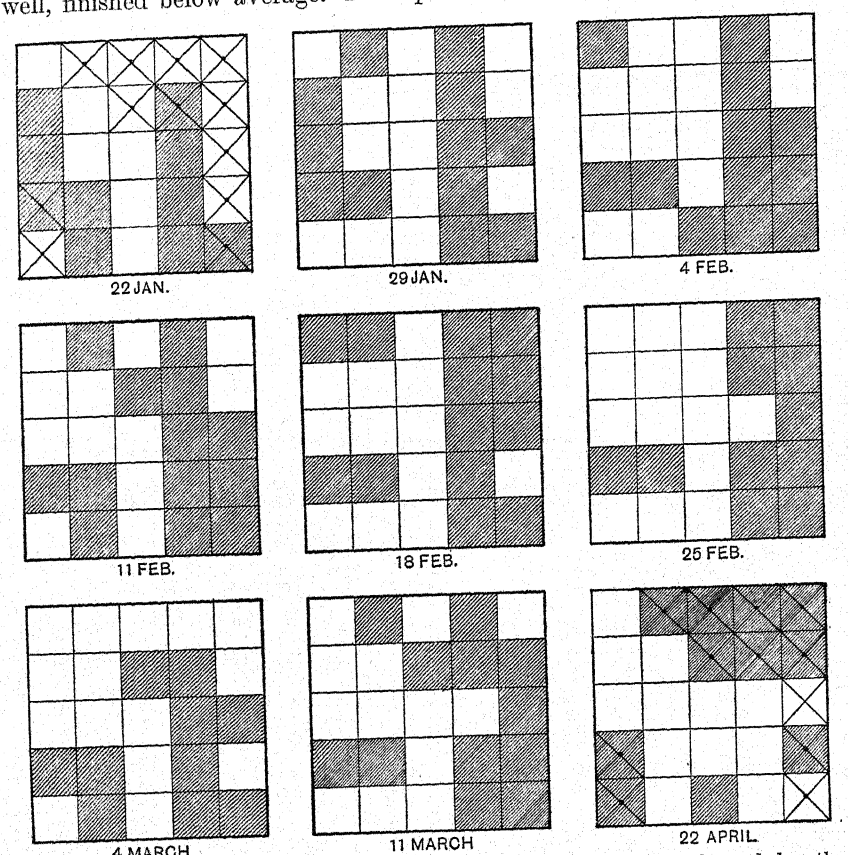


Diagram IV. A plan of the plots for every tiller count; date of count is shown below the plan; every plot which exceeds in average number of side tillers per plant the mean of the combined plots of its variety, is shaded; on the first and last plans are shown (×) the plots which at harvest exceeded in average number of ears per plant the mean of the combined plots of the variety; 1924–5 data.

Results of the same general character were obtained in the remaining three seasons of the experiment.

It is of much interest to examine, in the manner employed above, the behaviour of the separate wheat varieties. In all four years there is evidence strongly suggestive of varietal differences. That is to say, the

space distribution of the over-average plots is characteristic. Rivet wheat shows relative constancy of distribution of its over-average plots from week to week all through the period of early tillering. In the case of Squarehead's Master, there is less constancy. The distribution for Yeoman is characteristically erratic in all four seasons. This behaviour harmonises, broadly, with what is known of the dependability of these wheats under a diversity of conditions. A fresh line of study in varietal comparison, possibly worth close attention, is thus opened up.

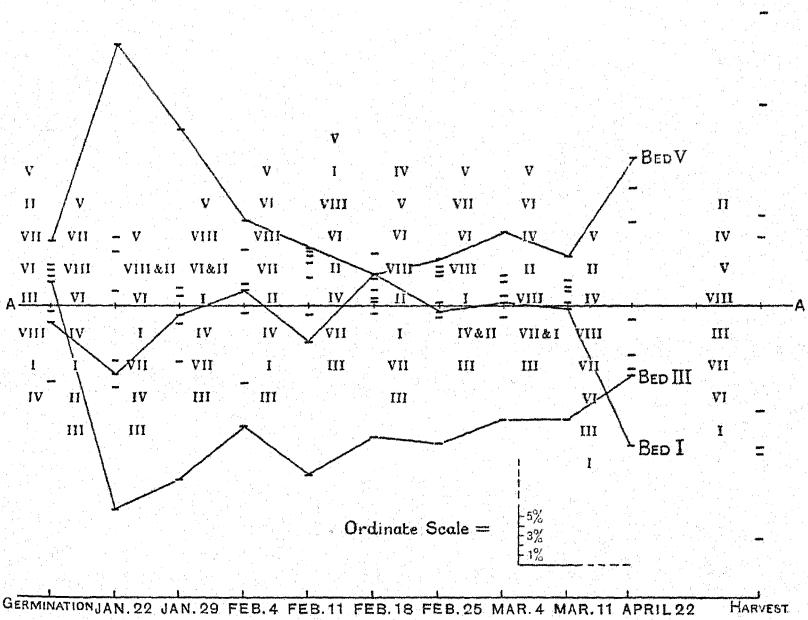


Diagram V. Showing the relative order (judged by average number of side tillers per plant) of the eight beds of variety *A* in 1924–5.

Closer scrutiny of soil heterogeneity may be made by examining the relative progress or plot history of the individual plots of each variety in turn. Diagram V affords an illustration for Squarehead's Master (variety *A*). The data presented are for germination percentage, for the nine tiller counts already dealt with, and for number of ears per plant at harvest. Variety average (for the combined eight plots) is taken as 100 (*AA*) and the average value for every bed is expressed in terms of this. Bed values are marked on the ordinate pertaining to every count. To identify bed values the serial numbers of the beds (I to VIII) are written in the order of magnitude of their mean values to the left of every

ordinate. Magnitude of inter-bed differences may be read off by use of the ordinate scale shown on the diagram.

The extent to which order of relative development fluctuates from week to week, among the eight beds, may be perceived from the manner in which the bed serial numbers I to VIII fluctuate. Disregarding small interchanges of position certain facts may be noted. Bed V is consistently best developed throughout early tillering (with a small exception on February 25). Bed III, germinating well, is thereafter most backward of all in development up to April 22. In Bed I the sudden drop during the period March 11 to April 22 is remarkable. The fairly close similarity in bed order between tillering on April 22 and ear formation at harvest is of distinct interest.

Soil heterogeneity upon the plot area shows, on the whole, a fair time-constancy. There are, nevertheless, certain striking examples of inconstancy as, for instance, in Bed I. It may well be supposed that soil conditions on a plot may be relatively favourable to plant development at one time and unfavourable at another. Moreover, if the populations of two plots, by reason of their environments, are markedly different in development at one time, they must have, thereafter, very different experiences. A subsequent spell of weather finds them in different physiological phases and thus may react very differently upon them. The bed of poorer early development may at some later stage be favoured more markedly than the other.

APPENDIX I.

THE EXTENT OF DAMAGE BY WHEAT MIDGE (*CECIDOMYIA TRITICI, et al.*) AND WHEAT BULB-FLY (*HYLEMYIA COARCTATA*).

In East Anglia 1925–6 was an exceedingly bad wheat year. There were many severe attacks of wheat bulb-fly. These, as usual, were mainly where wheat followed a fallow and in not a few cases the crop had to be ploughed up. The varietal comparison plots (Section III) were severely affected and from the close observations made it is possible to measure the ravages of this pest. But the worst feature of the season was disappointment in threshing. A well-known newspaper correspondent wrote (in July), "the fields are full of ears"; and he voiced the general expectation of heavy yields. In carrying out hybridisations towards the end of June, the larvae of wheat midges (mainly *C. tritici*) were observed in unusual abundance. Watch was therefore kept upon the pest, and large random samples of ears were drawn from field crops

at harvest. Many fields were practically unaffected, but many were severely attacked. Counts were made of the numbers of sound and affected grains in every ear of the samples. Grains attacked by midge larvae are completely and characteristically shrivelled. They are smaller even than tail corn and thus entirely useless. It is possible that certain shrivelled grains represented the action of corn thrips or some agency other than wheat midge, but it is believed that the proportion of these was very small. Farmers complained that they "threshed all day for twenty sacks" in spite of putting great quantities of straw through the drum. In the heap from the "dirt and weed seeds" spout of the drum, midge larvae were often the main constituent. Samples from twenty definitely affected fields proved to have an average of 20–30 per cent. of shrivelled grains. And it was a percentage deficiency, of this order, below average yield, that trustworthy farmers and enginemen (working the travelling threshers) reported as typical of the crops of the season.

Details for the ear sample from one field, given in Table XXV, will serve to illustrate typical facts. For the whole sample the percentage of shrivelled grains was 22·7. There is an evident association between ear size (number of grains per ear) and percentage of shrivelled grains. The smallest ears are markedly the most affected. These ears represent in general the later side tillers of plants, and emerge a few days after the ears of the main axes and earlier side tillers. It is probable that they emerged when the egg-laying adult midges were most abundant, and that the earlier ears largely escaped. Considerable differences in infection, corresponding to comparatively small differences in time of ear emergence, have frequently been noticed on small plots where a wide diversity of varieties was growing. Similarly, Rivet wheat, later in ear emergence than any other form grown in England, has appeared to be remarkably free from attack.

Table XXV. *Frequency distribution of number of grains per ear and of percentage of shrivelled grains for a random sample of ears from a field crop; Wilhelmina wheat,* 1926 *crop.*

No. of grains per ear	Frequency (%) of ears	Percentage of shrivelled grains
0– 9	0·76	—
10–19	3·05	33·3
20–29	6·10	31·2
30–39	14·50	31·0
40–49	14·50	22·3
50–59	17·55	22·8
60–69	23·65	21·4
70–79	16·79	20·8
80–89	3·05	17·4

These estimates of damage suggest that the low yields frequently reported in East Anglia in 1926 were primarily attributable to wheat midge. And further, that to this long familiar pest must be ascribed far more serious damage than has been commonly supposed.

The general course of the wheat bulb-fly attack remains for consideration. Its essential features are set out in Table XXVI, and the effects on development may be judged by comparing the 1925–6 results with other years in Tables II and IV. Sowing, as in the other years of the experiments, was on November 5. Up to two months after sowing, plant survival was about as in other years. Casualties were such, however, as to induce losses of from 55 to 32 per cent. (of seeds sown) in the mature populations.

Table XXVI. *The salient features of the wheat bulb-fly attack in* 1925–6 *for varieties* A, B *and* C.

	A	*B*	*C*
Percentage of seeds sown which survived up to 2 months after sowing	87	87	87
Percentage of plants surviving at 2 months after sowing which formed ears at harvest	78	66	52
Percentage of seeds sown which gave (ear-bearing) plants at harvest	68	58	45
Average number of side tillers per plant on Mar. 11	1·27	0·85	0·58
Average number of side tillers per plant on Apr. 22	3·40	4·04	3·80
Percentage of plants found to be affected on Mar. 11	79	84	88
Average number of side tillers per affected plant on Mar. 11	1·09	0·04	0·72
Average number of side tillers per non-affected plant on Mar. 11	1·94	1·80	1·57
Average number of ears per plant at harvest	4·43	4·62	4·01

Germination was not complete until early January, being much retarded by frosts. By mid-February bulb-fly damage was readily apparent. Many plants lost the main axis before any side tillers had come into view and then, at varying intervals, side tillers arose from these apparently "dead" plants. In time, varying numbers of the side tillers were observed to be affected. Thus "tillering" was severely checked. This is clearly shown in Table II. For instance on February 18 tillering was about the same for 1925–6 as for 1927–8, but by March 11 the 1925–6 values were relatively very low, especially in varieties *B* and *C*. In all the early stages of growth bulb-fly damage was heaviest in *C* (Rivet), next in *B* (Yeoman), and least in *A* (Squarehead's Master). In percentage of affected plants on March 11 the $A \sim C$ difference alone is actually significant.

The plots looked at their worst on March 11 and Table XXVI shows the great proportion attacked by that date (roughly 80 per cent.) and the marked difference in tillering between affected and non-affected

plants. It must be pointed out that the data cannot have been entirely dependable. For certain plants may have been slightly attacked in early February, but have shown no remains of dead tillers on March 11.

A period of drought came to an end on April 5 and from then onwards, in plants able to survive, growth was rapid. The tillering on April 22 (Table XXVI) gives evidence of this. In varieties *B* and *C* recovery has been especially marked. This accords with the general tillering capacity shown by these varieties, as compared with *A*, in other years.

Actual extinction, the culmination of a long struggle between tiller formation and the devouring larvae, was most common in the period March 15 to May 15. In normal cases exceedingly small proportions of plants die during this time.

As Table II shows, the average number of ears per plant at harvest was higher than in any other year. Heavy casualties greatly increased effective spacing with the result, always found, that both tillering and survival of tillers to harvest were abnormally high. But the ear-bearing tillers were formed later than in the other seasons. In consequence (*vide* Table IV) yield per ear was extremely small. Finally, yield per unit area (*vide* Table IV) showed a reduction of 25–40 per cent. on other years. It may be surmised, therefore, that the yield loss induced by wheat bulb-fly was of the order of fully 30 per cent. Having in mind the favourable conditions of plots in contrast with fields—discussed in Section V—more severe losses would probably have to be expected in an ordinary field crop.

The geographical incidence of attack was studied by evaluating correlations between adjoining beds, and by marking on a plan the positions of all affected plants. There was no evidence of localisation of attack.

APPENDIX II.

THE EFFECT OF DROUGHT ON THE DEVELOPMENT OF WHEAT.

In 1928–9 a study of the influence of height of water table on plant development was commenced in a wheat field on gravel soil over gault clay. Ordinarily, at low-lying points in this field, the water table is almost at the surface in late winter. In summer the field is always very dry. The dry winter, followed by the marked drought of spring and summer, wrecked the experiment which was, indeed, transformed into a study of the effects of drought. All crops on light land in 1928–9 were very "patchy," development being fairly good at one point and exceedingly poor near-by. This sharply localised heterogeneity was particularly

marked on the field in question. By digging a number of trenches it was shown that depth of soil was definitely related to plant development at any point. The layer of top soil was extremely irregular in depth, changing swiftly from point to point between limits of about 3 and 37 inches. Between the top soil and the clay substratum was entirely unmodified river gravel. Irregularities of the kind here described characterise all the gravel soils to the west of Cambridge.

Certain foot-lengths of drill row had been under observation from sowing time onwards so that developmental records were available. At harvest, in an area of 24 yards by 10 yards (one of the blocks or units of observation of the original experiment), the foot-lengths were classified as showing "good" or "bad" plant development. On these the plants were dug up and counted for ear number, ear size, etc., and the depth of the top-soil layer was determined.

In 1924–5 this same field, being under the same wheat (Little Joss), was used in a study of development and yield and the census method (foot-length samples) was adopted (Engledow (3)). It is therefore possible to compare development in 1924–5 with development in 1928–9, two degrees of water shortage being observed in the latter season (viz. "good" foot-lengths on relatively deep top soil and "bad" foot-lengths on shallow top soil). The essential data are given in Table XXVII.

For the 1924–5 crop a low seed rate was used and number of plants per foot was therefore far less than for 1928–9. This does not, however, disturb the general comparison which is to be drawn. Other factors apart, the data of Table XXVII are based, in effect, on three degrees of water supply. The year 1924–5 was more or less "normal"; on the "good" feet of 1928–9, with an average top-soil depth of 17·3 inches, there was partial drought; while the "bad" feet, with an average of 7·4 inches top soil, represented (for this country) severe drought. Over the period Oct.–Aug., 1928–9 had 3·31 inches less rain than 1924–5, but its important peculiarity was a very low fall in Jan.–March (1·76 as against 4·04 inches in 1924–5). This low supply was adequate for the crop in the period Jan.–March as the average tillering in April attests. But it was insufficient to provide a storage of water for the ensuing months of rapid growth and higher temperature. It is naturally, therefore, in development from April onwards that drought effects are apparent. Between the top soil of this field and the clay below is a coarse river gravel which, while often saturated in winter, becomes later an insulating layer. It does not readily lift water from the clay to the top soil. In consequence, depth of top soil at any point largely determines adequacy of water supply to the crop at that point.

Ordinarily, such wheat plants as die become casualties before April, and by harvest their remains are no longer intact. In 1928–9, however, a number of plants died late on in the season from drought. These were the earless plants mentioned in the table.

In 1928–9 early tillering—up to mid-March—was much retarded by severe and prolonged frost. With warmer weather it swiftly accelerated and, despite the much denser plant population, had by mid-April reached a value greater than for 1924–5. This value was the same on good and bad feet.

Table XXVII. *Comparative development of wheat* (i) *in* 1924–5, (ii) *on "good" foot-lengths in* 1928–9, (iii) *on "bad" foot-lengths in* 1928–9.

	1924–5	1928–9 "good" foot-lengths	1928–9 "bad" foot-lengths
Rainfall (inches) October	3·71	2·39	
November	2·25	1·50	5·92
December	1·08	2·03	
January	0·81	1·25	
February	1·97	0·48	1·76
March	1·26	0·03	
April	1·25	1·25	
May	2·04	2·81	4·86
June	0·50	0·80	
July	1·87	2·37	2·74
August	1·85	0·37	
Total	18·59	15·28	
Av. no. of plants per foot at harvest	11·2	21·8	20·5
Av. no. of side tillers per plant (mid-April)	4·8	5·2	5·2
Av. no. of earless plants per foot at harvest	nil	0·61	2·45
Av. no. of ears per plant (excluding earless plants)	1·85	1·55	1·01
% survival rate of April tillers to form ears (excluding earless plants)	38·3	25·0	16·3
% of infertile florets per ear	(not counted)	29·5	47·9
Av. no. of spikelets (total) per ear	(not counted)	19·9	15·0
Av. depth of top soil (inches)	—	17·3	7·4
Coefficient of correlation:			
% sterile florets : depth of top soil	—	—	−0·42
No. of plants per foot : no. of side tillers per plant (mid-April)	−0·47	−0·55	−0·55
No. of plants per foot : no. of ears per plant (harvest)	−0·66	−0·54	−0·27

Drought effects are patent in tiller survival. For the three "degrees" of drought to which Table XXVII relates the proportions of April tillers which survived to give harvest ears were 16·3 per cent., 25·0 per cent., and 38·3 per cent. The corresponding average numbers of ears per plant were 1·01, 1·55, and 1·85. Size of ear, measured by number of fertile spikelets, was strongly affected. No data are available for 1924–5, but for the good and bad feet of 1928–9 the proportions of infertile spikelets

were 29·5 per cent. and 47·9 per cent. Not only degree of fertility, but actual number of spikelets formed, was affected by drought, the averages per ear for 1928–9 being 19·9 on good and 15·0 on bad foot-lengths. The primordia of the florets were laid down in April when water shortage was just becoming effective. It is for this reason that in respect of number of spikelets formed per ear, good and bad feet differ far less than in percentage of infertile spikelets. The close connection between drought (as here used) and fertility is shown by the correlation $r = -0{\cdot}42$ between percentage of sterile florets and depth of top soil.

The correlations shown in the bottom two rows of Table XXVII indicate that in 1928–9 spacing affected tillering up to April in normal fashion: but by harvest, in extreme drought (bad feet) this influence was abnormally low.

In East Anglia spring droughts are characteristic. The nature of their influence on cereal yield is outlined by these data which point out fertility of florets as perhaps the most vulnerable yield component.

In the ultimate form this experiment was given, the whole of the collection of data and compilation was in charge of Mr G. D. Stevenson, of the School of Agriculture, to whom the authors are much indebted for careful work and constructive ideas.

A RÉSUMÉ.

I. A GENERAL INTRODUCTION.

The solution of what is defined as the "English cereal yield problem" lies partly in plant breeding and partly in improvement of the practices of husbandry. Fundamental advances in both of these depend upon an analysis of yield. Limitations of knowledge constrain the form of analysis, making it essentially a comparison of varieties under different environments. Earlier studies have demonstrated that such comparison must be on a developmental basis; that rate of tiller formation is a facile and good index to development besides being closely related to yield; and that among external or environmental factors which govern yield in field crops, fluctuating spacing is of high importance. Further, there occurs in tillering a critical period. This marks the time up to which spacing does not influence tillering, and before which are formed those tillers that survive to harvest and bear ears.

The studies here recorded are essentially a comparison of three wheats at different spacings including ordinary field sowing. Development is traced by periodic observations on tillering and special attention is given to the critical period. A life-history of the wheat plant is outlined from

the combined results of various experiments and subsidiary studies are made upon germination, inter-variety competition, and certain forms of parasitic damage.

II. An outline of experimental procedure.

Details of sowing, lay out, and observations are given for the experiments of the series. The wheats used, A = Squarehead's Master, B = Yeoman and C = Rivet, are described. Symbols are adopted for tillering, namely, T_0 = main axis of the plant, T_1 = first side tiller, etc.

III. Comparative development and yield in three wheat varieties during four seasons.

Relative yielding capacity is of very different provenance in the three varieties, and the results disclose the subtle manner in which features of development influence final yield. Throughout the four-year period the varieties maintain a constant order in respect of thirteen major and certain other plant characters. Thus, for example, the order is $B > A > C$ for rate of germination, percentage of germination, plant survival, rate of leaf development, rate of early tillering, and ear formation.

Early tillering is shown to be an important index to yield. The exact details of its chronology constitute a definite characteristic of every variety. It must be ranked as one of the chief plant characters, *i.e.* internal yield factors.

The experimental results are closely conformable with and interpretative of the behaviour of the varieties in ordinary wheat-growing practice.

IV. The influence of spacing on the development and yield of the three varieties.

With spatial change the varieties preserve the same relative order of magnitude in tillering and other attributes as was found in the single-spacing experiment of Section III. There are, nevertheless, important differences in varietal response to spacing. This is particularly true of size of side tiller ears compared with the main axis ear. The effect derives interpretation from what is shown of the influence of spacing on the critical period of tillering. For a field crop, that is, a complex of spacings, the two aspects or phases of the critical period are practically synchronous. In the spacing plots their time-incidences prove to be separable by influences of weather, and the extent of the separation is definitely varietal. Variety behaviour is such as to result in an association between numerous ears per plant and relatively small side tiller ears. Such an

association would naturally limit the possibilities of breeding for higher yield by trying to combine in one variety high ear formation and great ear size. This difficulty may be met, it is suggested, by ensuring high soil fertility during early development. This is tantamount to postulating that if wheats of unusually high potential yielding capacity can be had, they will display their superiority only under "high farming."

Spatial influence on yield per unit area, as here measured, fails to show agreement with results from studies of field crops. This problem is discussed in Section V.

V. Varietal development under field conditions and a comparison between spatial response on the plot and in the field.

Under field conditions the development of varieties, one relatively to another, closely resembles what is found on plots. But for all varieties development and yield differ sharply under the two sets of conditions. In the field tillering, ear formation, ear size, and yield per unit area, attain much lower values than on plots. Further, the response of tillering to spacing is far less marked. The explanation of these effects is believed to lie in the superior cultural conditions of the plot. Of these, regularity of sowing, as to distance between seeds and as to depth, is considered to be the most important.

VI. Inter-plant and inter-variety competition.

Two investigations were made upon competition. In the first, varieties *A* and *B* were sown together (one seed of each in every hole), and also in alternate rows (two seeds of the same variety in every hole of the row). Development and final yield were such as to suggest no significant degree of variety interference.

A second investigation consisted of periodic removal of two plants out of every three in the row. The first thinning out was made just after the critical period of tillering. All the thinnings, but particularly the early ones, induced extra tillering. But the extra tillers did not fructify, and at harvest all the treatments gave about the same yield as the unthinned control.

VII. The germination and plant survival of wheat.

Of seeds sown there normally survive to harvest-plants some 60–90 per cent. on experimental plots and 50–80 per cent. in the field. Most of the casualties occur within three months of sowing. Tests show that upon very rough tilths germination may be very low, particularly with

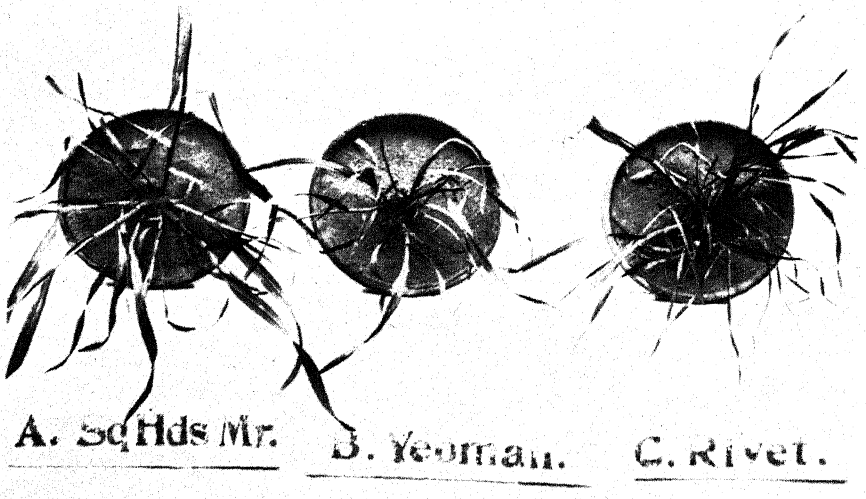


Fig. 1. Typical (modal) plants of the three varieties lifted from the plots and potted (from Exp. 1). In *A*, side tillers are fairly numerous and well developed; in *B* they are more numerous but many are extremely small; in *C* they are extremely numerous but many are diminutive. Some 60 per cent. of the tillers here shown—for all three varieties—died before harvest.

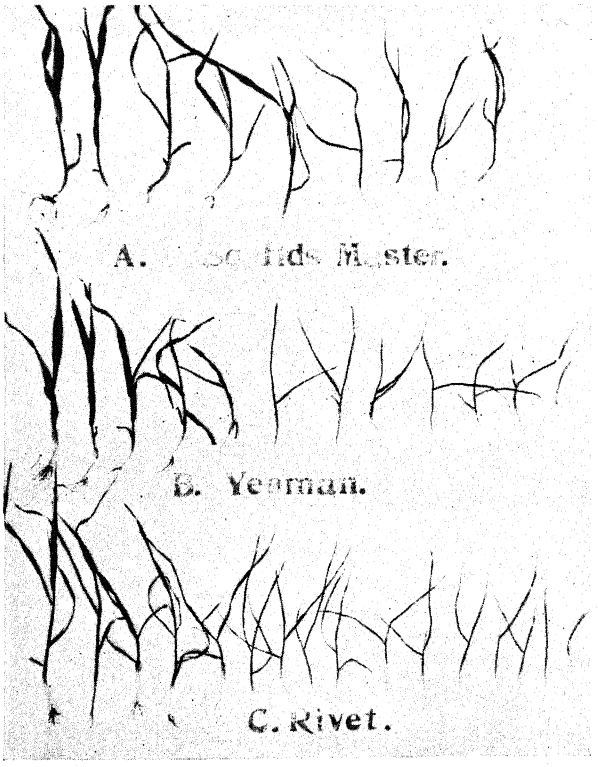
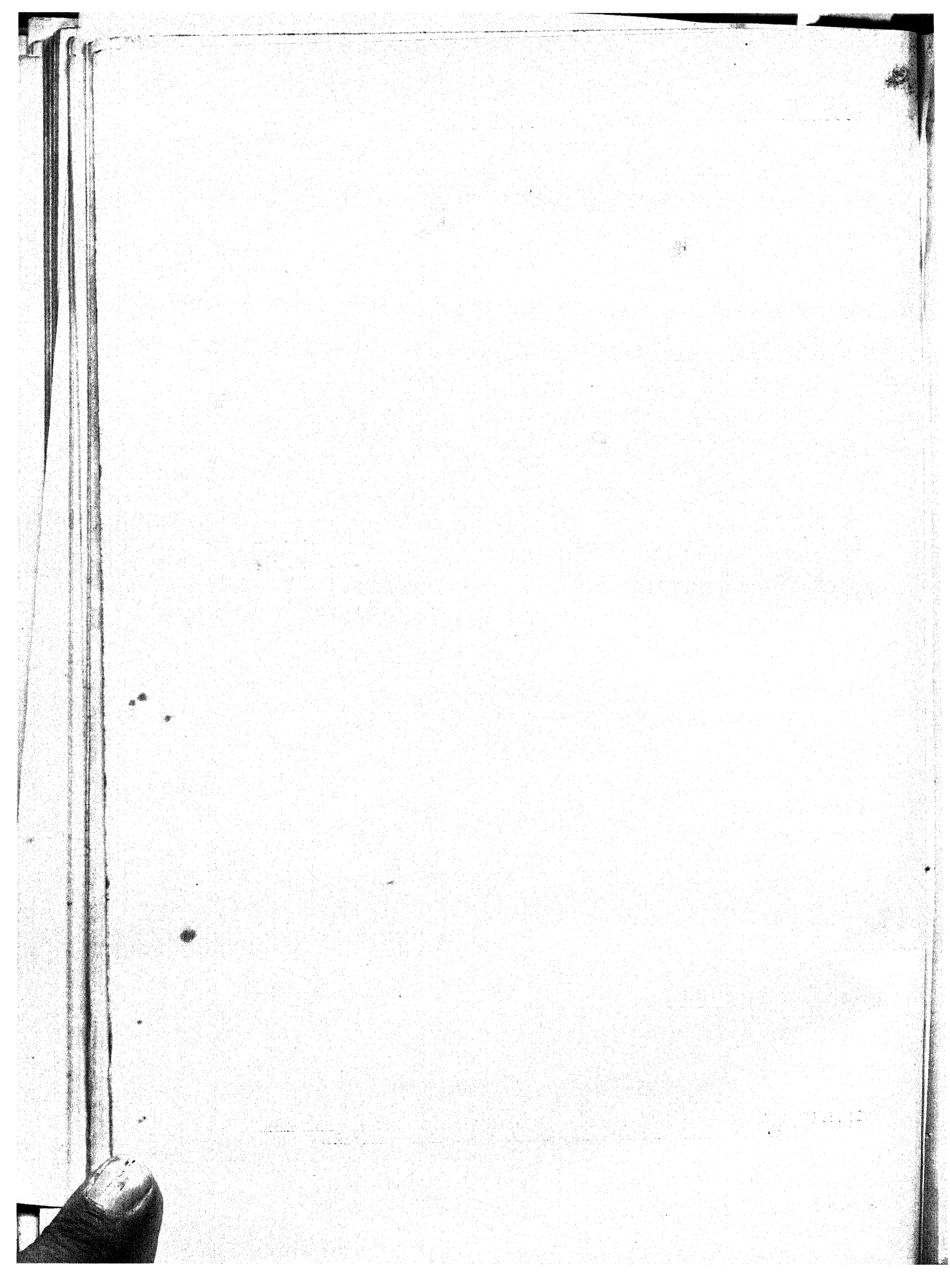


Fig. 2. The typical plants depicted in Fig. 1 are here shown dissected into their component tillers. The varietal differences explained in the legend below Fig. 1 stand out clearly here.



"bad" seed. Thus samples of 90 and 80 per cent. laboratory germination gave respectively 65 and 32 per cent. on a very rough field tilth. In practice "bad" seed is physiologically imperfect, being either immature or damaged by wetness. From such seed, on heavy land, there not uncommonly arise heavy losses of potential yield.

Rivet wheat is characteristically of lower germination capacity and more tardy in germination than Squarehead's Master or Yeoman.

Seeds which germinate late grow into plants of abnormally low yield. On some field sowings the yield loss from defective late germinating seeds may be 15–20 per cent.

Rate of germination is largely controlled by the accumulated temperature of the first few weeks after sowing.

VIII. A CONSPECTUS OF THE DEVELOPMENTAL HISTORY OF THE WHEAT PLANT.

From the data of foregoing sections and of earlier studies of this series there is compiled a chronology of the life history of the wheat plant as grown in East Anglia. The course of tillering, ear formation, grain filling and other aspects of growth, is traced. It is prepared as a guide to investigations on external yield factors such as parasitism and fertilisers.

IX. WEATHER AND PLANT DEVELOPMENT.

Relationships are suggested between the progress of tillering and two aspects of weather, namely, rainfall and accumulated temperature.

X. SOIL HETEROGENEITY AS REFLECTED BY PLANT DEVELOPMENT.

Development, measured by tillering, is traced out for the individual plots of one of the experiments. If plant development be a fair reflection of soil heterogeneity, then the distribution of heterogeneity over the plot area is not constant throughout the season. Final plant development on a plot is closely related to development at the end of April.

APPENDIX I. THE EXTENT OF DAMAGE BY WHEAT MIDGE (*CECIDOMYIA TRITICI, et al.*) AND WHEAT BULB-FLY (*HYLEMYIA COARCTATA*).

In 1925–6 the very low yields on many wheat fields were mainly attributable to wheat midge. Careful estimates disclosed losses of 20–30 per cent. There was a severe wheat bulb-fly attack on one set of plots in the same season. Its incidence, the plant mortality, and plant recovery were measured. Some 80 per cent. of the plants were attacked, from 30 to 50 per cent. of the seeds sown failed to give harvest plants, and the yield loss was of the order of 30 per cent.

Appendix II. The effect of drought on the development of wheat.

By comparing the development and yield of a wheat variety on the same field in two very different seasons, the influences of drought upon growth stages are illustrated.

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(*Received February 17th*, 1930.)

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NOTE ON THE OCCURRENCE OF ELEMENTARY CARBON IN SOILS.

BY G. W. ROBINSON, M.A., AND W. McLEAN, B.A., B.Sc.
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IN the course of determinations of organic carbon in soils, some unexpectedly high figures suggested the possibility that free carbon in the form of fragments of coal or cinder might affect appreciably the results obtained. The soils in question were from the Coal Measure district of Flintshire, and the presence of notable amounts of coal was not surprising. Even in soils from other districts, however, it is not unusual to find small carbonaceous fragments, which are generally noticeable in the coarse sand separated in mechanical analysis. Their presence is not difficult to explain, for domestic ashes containing unburnt coal or cinders are often thrown on to the manure heap, and thus get carted on to the soil where they persist unaltered. The highest proportions of elementary carbon may be expected in heavily manured soils such as those of gardens.

It is obvious that organic carbon becomes an untrustworthy measure of the organic matter if it consists to an appreciable extent of elementary carbon, and it is thus necessary to consider how a correction may be applied. Simple removal of carbonaceous fragments, even from a small sample, is a laborious and uncertain procedure, for where there are fragments large enough to occur in the coarse fractions, there will also be fragments of finer grade which will escape detection and removal. The possibility suggested itself that, by choosing an appropriate settling velocity, it might be possible to remove the organic matter completely by decantation, and to leave all or nearly all the elementary carbon in the residue. By determining the amount thus left, it should be possible to obtain a correction to be deducted from the total carbon found in the original soil. A simple mechanical analysis was therefore made, using a settling velocity of 10 cm./250 sec., on two 100-mesh samples of coal and coke respectively. The following proportions of the 100-mesh sample were found in the residue after decanting with the above settling velocity.

	Percentage in residue
Coal	66
Coke	84

Assuming that the carbonaceous residue in soil is partly coal and partly coke or cinder, we may take 75 per cent. as a first approximation for the proportion left in the residue after decantation. We should prefer in future work to use the International time/depth ratio 10 cm./288 sec. The results could scarcely differ appreciably and it might be possible to combine the above procedure with mechanical analysis, by retaining the two sand fractions for a carbon determination.

A number of soils (100-mesh sample) were now peroxided and sedimented, using the above settling time and depth. The residue in each case was dried and submitted to an organic carbon determination. The amount of organic carbon in the elementary state was obtained by dividing the result found by 0·75. This figure was then used as a correction to be applied to the total organic carbon found in the original soil. The results are shown in the following table.

Table I. *Percentage total organic carbon and elementary carbon in certain soils.*

	Organic carbon uncorrected	Carbon in residue *A*	Elementary carbon *A*/0·75	Organic carbon corrected
	(%)	(%)	(%)	(%)
Coal Measure soil, Flintshire	3·94	0·97	1·29	2·65
,, ,,	4·20	1·14	1·52	2·68
,, ,,	5·64	1·03	1·37	4·27
,, ,,	2·35	0·08	0·11	2·24
Garden soil, Flintshire	6·45	1·36	1·81	4·64
Garden soil, Anglesey	5·31	0·76	1·01	4·30
,, ,,	4·10	0·42	0·56	3·54
Garden soil, Caernarvonshire	5·84	1·30	1·73	4·11
,, ,,	8·56	1·36	1·81	6·75
,, ,,	4·43	0·55	0·73	3·70
,, ,,	4·52	1·21	1·61	2·91
Barnfield Rothamsted F.Y.M. plot	3·13	0·20	0·28	2·85
Upland Grass Heath, Caernarvonshire	8·04	0·02	0·03	8·01
Pasture soil, Anglesey	5·07	0·08	0·10	4·97
,, ,,	4·12	0·18	0·24	3·88

It will be noticed that in some cases the correction for elementary carbon amounts to a considerable proportion of the uncorrected carbon figure. The use of uncorrected figures for organic carbon in studies on soil organic matter can thus be the cause of considerable error, and where the presence of elementary carbon is suspected, the correction should be determined and applied. The negligible figure for elementary carbon obtained in the case of uncultivated mountain soil shows that

the decantation used has been successful in removing all, or nearly all, the true organic matter, and that the carbon actually found in the residue may be assumed to be elementary carbon. The uncertainty lies in the use of the factor for obtaining the elementary carbon from the residual carbon; but, unless the organic matter carbon is very low and the elementary carbon high, the error involved is of a lower order of magnitude than the content of organic carbon.

(*Received February 28th*, 1930.)

THE CARBON-NITROGEN RATIO OF SOIL ORGANIC MATTER.

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During recent years the carbon-nitrogen ratio of soil organic matter has engaged the attention of a large number of investigators. An excerpt from *Soil Conditions and Plant Growth* by E. J. Russell, given below, will indicate the nature of the conclusions arrived at. Referring to the activities of micro-organisms under the heading "The effect of adding plant residues to the soil," Russell states: "The net results of these various activities is that the ratio of carbon to nitrogen in a soil is not easily disturbed; in humid conditions it is about 10. Addition of non-nitrogenous organic matter such as starch, cellulose, or straw (which is poor in nitrogen) sets in train the process of nitrogen fixation which increases the amount of nitrogen at the expense of a much larger amount of carbon, and also the microbiological assimilation of nitrates, which, while reducing the amount of carbon, protects the nitrogen already present against loss. Both sets of processes decrease the carbon and increase the nitrogen, thus restoring the original ratio. Addition of nitrogenous organic matter would increase the proportion of nitrogen, but it sets going a loss of gaseous nitrogen and also nitrification which produces nitrates easily lost. These losses of nitrogen proportionately exceed those of carbon, so that again the original ratio is restored."

With a view to ascertaining to what extent the C/N ratio approximates to the figure 10 : 1, the writer has determined this ratio for fifty British soils and sixteen foreign soils. The method used for the determination of organic carbon was the Kjeldahl method as worked out in this laboratory by Robinson, McLean, and Williams(1). The nitrogen was determined on the residue after the oxidation of the carbon in the Kjeldahl process, using a direct distillation method and employing 0·02 *N* acid and alkali with methyl-red as indicator. The great majority of the samples was done in duplicate, and in every case the duplicates showed practically complete agreement.

For economy of space the individual results are not set out in detail, but the range of values obtained for the C/N ratio may be seen in the following table.

Table I. *C/N ratios for fifty British soils.*

Range of C/N ratios	No. of soils	Range of C/N ratios	No. of soils
6·5–7·4	1	10·5–11·4	10
7·5–8·4	2	11·5–12·4	5
8·5–9·4	10	12·5–13·4	3
9·5–10·4	18	13·5–	1

On account of their special interest, the results for foreign soils are set out individually in Table II.

Table II. *C/N ratios for certain foreign soils.*

Soil	
Paddy soil, Siam, B.A.S. 9	11·6
Paddy soil, Siam, B.A.S. 7	11·4
Paddy soil, Siam, B.A.S. 5	11·3
Paddy soil, Siam, B.A.S. 3	9·0
Black turf soil, Transvaal	14·2
Red loam soil, Transvaal	14·4
Black soil, Kenya	15·1
Red doleritic soil, Natal	14·7
Gezira clay, Sudan A	23·0
Gezira clay, Sudan B	19·0
Lateritic soil, Samoa	20·7
Lateritic soil, Cuba	17·5
Alkali soil, California	8·8
Saline soil, California	2·0
Red clay, Trinidad	5·3
Black clay, Trinidad	10·4

The average C/N ratio for the fifty British soils works out at $10{\cdot}2 \pm 0{\cdot}3 : 1$, and the results form a normal frequency curve with a well-defined maximum at 9·5–10·4 : 1, although the range is fairly considerable, viz. 6·5–13·5 : 1. In view of this spread, it would obviously be out of the question to judge the percentage of organic carbon in the soil from the percentage of nitrogen, as was suggested by Fraps(2). This point is further emphasised when one examines the ratios for foreign soils, which are seen to vary from 2·0–23·0 : 1. The extremely low figure for the Californian saline soil is to be noted. Without further study, however, it is impossible to estimate the significance of this result.

Considering the extreme variations found in the C/N ratio, it occurred to the writer that useful information might be gained by an examination of the factors which would be likely to influence these ratios. These are dealt with in turn.

Regional effect.

It was noticed at an early stage in this investigation that soils from a limited area gave C/N ratios which varied only to a small extent. Table III will serve to demonstrate this point. Figures for the

percentages of carbon and nitrogen are also given, in order to show that, whilst the C/N ratios, for example, of several soils in one area are similar, the percentages of carbon and nitrogen may vary widely.

Table III.

Soil	Percentage		C/N ratio
	Carbon	Nitrogen	
Madryn 1, Caernarvonshire	3·85	0·34	11·3
Madryn 2, Caernarvonshire	4·31	0·39	11·1
Llysfasi A, Denbighshire	2·33	0·24	9·7
Llysfasi B, Denbighshire	4·17	0·44	9·5
Leadbrooke A, Flintshire	2·24	0·20	11·2
Leadbrooke B, Flintshire	2·09	0·18	11·6
Leadbrooke C, Flintshire	2·40	0·21	11·4
Pengoch Hill, Flintshire	2·56	0·23	11·1
Maesydre, Flintshire	2·68	0·24	11·2
Soil 92, Herefordshire	2·42	0·23	10·5
Soil 26, Herefordshire	1·71	0·19	9·0
Soil 139, Herefordshire	1·62	0·19	8·5
Soil 331, Herefordshire	1·32	0·15	8·8
Paddy soil, Siam, B.A.S. 9	6·28	0·54	11·6
Paddy soil, Siam, B.A.S. 7	6·37	0·56	11·4
Paddy soil, Siam, B.A.S. 3	1·63	0·18	9·0
Paddy soil, Siam, B.A.S. 5	3·4	0·30	11·3
Black turf soil, Transvaal	1·42	0·10	14·2
Black loam soil, Transvaal	1·87	0·13	14·4
Red doleritic soil, Natal	4·25	0·29	14·7
Gezira clay, Sudan A	0·46	0·020	23·0
Gezira clay, Sudan B	0·38	0·020	19·0
Lateritic soil, Samoa	3·31	0·16	20·7
Lateritic soil, Cuba	1·05	0·060	17·5

The bracketed soils are from the same area and show small variations in the C/N ratios, suggesting that these ratios are specific for each region. With some exceptions, soils in warm climates appear to have higher values than those in cool climates. This is contrary to the findings of Jenny (3), who states that the ratio tends to become wider (12 : 1 or 13 : 1) in colder climates and narrower in warmer climates (9 : 1).

Arable *versus* Grass.

To ascertain whether the C/N ratio varied according as the land was under cultivation or in grass, twelve fields, namely, six arable and six grass, were selected from the College Farm, Aber, Bangor. About a dozen auger samples were carefully taken throughout each field to get a representative sample. The percentages of carbon and nitrogen and the C/N ratios are given in Table IV.

Although the number of samples is small, these results show that in a comparatively small area—in this case within a mile radius—there is

no appreciable difference in the C/N ratio of soils, whether arable or under grass. The carbon and nitrogen percentages, however, are appreciably higher in the grassland samples. This is to be expected in view of the greater decomposition due to aeration that takes place in soils under cultivation.

Table IV. *C/N ratios in grassland and arable.*

Field no.			Percentage		C/N ratio
			Carbon	Nitrogen	
2 *a*	Old pasture		3·97	0·40	9·9
2 *b*	Old pasture		3·55	0·37	9·6
T 12	Old pasture		3·26	0·39	8·4
B 12	Old pasture		2·71	0·31	8·7
6	Two years' grass		2·78	0·29	9·6
5	Two years' grass		3·02	0·31	9·7
		Average	3·22	0·35	9·2
3	Arable		3·08	0·31	10·0
9	Arable		2·73	0·27	10·0
16	Arable		2·97	0·38	7·8
14 *a*	Arable		3·16	0·35	9·0
B 8	Arable		2·66	0·27	10·0
14 *b*	Arable		2·95	0·33	8·9
		Average	2·93	0·32	9·2

POSSIBLE CORRELATION OF C/N RATIOS WITH FERTILITY.

A number of soils of known fertility was selected from widely different areas, and examined in order to ascertain to what extent the ratio varied with varying fertility as indicated by their known crop-producing capacity. The percentages of carbon and nitrogen and the C/N ratios are given in Table V.

Although the bracketed soils in Table V—those from the same area—indicate that soils in high condition have slightly higher ratios, yet there is not sufficient agreement in this respect to give any significant correlation between fertility and C/N ratios. The regional effect appears to mask any general correlation of this ratio with fertility. The extreme cases of the Rothamsted and Woburn soils, however, seem to indicate some correlation of C/N ratio with fertility.

On a further examination of Table V, however, it will be seen that, when soils from the same area are considered, the percentages of total carbon and total nitrogen are in every case higher, and significantly so in soils in a high state of fertility, than in poor soils. Cases could be cited of soils with very high percentages of carbon and nitrogen, but which are poor from the point of view of crop production, owing to

other operating factors such as lack of drainage, altitude, etc. The writer, therefore, suggests that the total organic carbon or the total nitrogen might be used as an index of fertility in the examination of crop-bearing soils in the same area, and under the same type of management and cultivation. For routine work, the carbon determination is to be preferred both for rapidity and ease of manipulation.

Table V. *C/N ratios of soils of varying fertility.*

Soil	State of fertility	Percentage		C/N ratio
		Carbon	Nitrogen	
Aber, T 12	High	3·26	0·39	8·4
Aber, 2	High	3·97	0·40	9·9
Aber, 6	Moderate	2·78	0·29	9·6
Aber, B 12	Low	2·71	0·31	8·7
Maesydre, Flintshire	High	2·68	0·24	11·2
Leadbrooke D, Flintshire	Low	2·34	0·20	11·7
Stackyard, F.Y.M. plot, Woburn	High	1·51	0·15	10·1
Stackyard, no manure plot, Woburn	Low	0·90	0·10	9·0
Broadbalk, F.Y.M. plot, Rothamsted	High	2·70	0·26	10·4
Broadbalk, no manure plot, Rothamsted	Low	1·06	0·12	8·8
Barnfield, F.Y.M. plot, Rothamsted	High	2·85	0·28	10·2
Barnfield, no manure plot, Rothamsted	Low	0·71	0·11	6·5
Herefordshire 92	High	2·42	0·23	10·5
Herefordshire 26	Low	1·71	0·19	9·0
Herefordshire 139	High	1·62	0·19	8·5
Herefordshire 331	Low	1·32	0·15	8·8
Paddy soil, Siam, B.A.S. 9	High	6·28	0·54	11·6
Paddy soil, Siam, B.A.S. 7	High	6·37	0·56	11·4
Paddy soil, Siam, B.A.S. 3	Low	1·63	0·18	9·0
Paddy soil, Siam, B.A.S. 5	Low	3·40	0·30	11·3

VARIATION IN C/N RATIO IN SOIL PROFILES.

The percentages of carbon and nitrogen and the C/N ratios for two profiles are given in Table VI.

Table VI.

Bracken profile, Aber	Percentage		C/N ratio
	Carbon	Nitrogen	
0–2 in.	15·91	1·14	13·9
2–6 in.	8·15	0·71	11·5
6–12 in.	5·38	0·47	11·4
12–18 in.	3·45	0·32	10·8
18–24 in.	3·12	0·28	11·1
24–30 in.	2·70	0·23	11·7
Vaccinium profile, Aber			
2–4 in.	11·89	0·72	16·5
4–10 in.	5·97	0·45	13·3
10–18 in.	3·72	0·29	12·8
18–24 in.	2·83	0·23	12·3
24–30 in.	1·85	0·16	11·6

It will be seen that there is a tendency for the C/N ratio to decrease in descending the profile, but whilst in the bracken profile a steady figure is revealed below the second inch, in the vaccinium profile the fall is maintained throughout. It should be added that the top 2 inches of the second profile was not examined. It consisted of a peaty matted layer which presented great difficulties in sampling. The high figure for the C/N ratio in the top layer of the profiles may be attributed to the presence of unhumified organic matter.

General discussion.

Various investigators have given the carbon-nitrogen ratio for soils as 10 : 1. Whilst this may be taken as a general figure for soils in Britain, it must be borne in mind that there is an appreciable variation from place to place, and this may be due to variation in climatic conditions, or to a combination of climate and other soil factors.

The C/N ratios for foreign soils vary widely, and it is probable that climatic conditions play a dominant part in determining the ratio for soils in any particular area. From the evidence presented, it seems that soils in warm climates have higher ratios than soils in cool climates, and that the C/N ratio is specific for each particular region.

Cultivated soils and those in grass, within limited areas at any rate, give approximately similar ratios, but the percentage of carbon and nitrogen is, on the whole, lower in the cultivated soils. In the case of soils showing extremes in fertility, such as the Rothamsted and Woburn farmyard manure and no manure plots, there is a decided difference in the C/N ratios—those in good condition giving the wider ratios. On the other hand, one fails to find any clear correlation of C/N ratio with fertility in soils farmed in a normal manner. There is, however, a distinct correlation between either the carbon or nitrogen percentages and fertility among soils of the same region. Soils in high condition give definitely higher percentages in carbon and nitrogen than poor soils in the same area. The higher carbon and nitrogen content may reflect the greater additions from plant residues in such soils. To some extent the effect may be due to more liberal manurial treatment.

Summary.

1. The average carbon-nitrogen ratio for fifty British soils from widely distributed areas approximates to the figure 10 : 1 given by other investigators. The range of variation is from 6·5 to 13·5 : 1. Sixteen foreign samples gave C/N ratios varying from 2·0 to 23·0 : 1.

2. Soils from limited areas, whether high or low in organic carbon, give approximately constant ratios, but these ratios vary from place to place according to soil, climate, etc. It is suggested that the C/N ratios may be specific.

3. The C/N ratios of arable soils do not differ appreciably from those of grassland soils. The percentages of carbon and nitrogen are somewhat higher in the grassland samples than in the arable samples.

4. There is no marked correlation between C/N ratios and fertility. The percentages of carbon and nitrogen are, however, in all areas examined, higher in soils of high fertility than in poor soils in the same area.

5. The writer suggests that the total organic carbon or the total nitrogen might be used as an index of fertility in the examination of crop-bearing soils in the same area. For routine work, the carbon determination is to be preferred both for rapidity and ease of manipulation.

6. The C/N ratio decreases in descending a profile. The high ratio in the top layer is, no doubt, due to the presence of plant remains.

The author's thanks are due to the Rothamsted Experimental Station, to Mr T. Wallace of Long Ashton, and to Major W. R. S. Ladell for furnishing certain samples for this investigation.

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(*Received February 28th*, 1930.)

NOTE ON THE DETERMINATION OF EXCHANGEABLE SODIUM IN SOILS.

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Until recently, no satisfactory direct method has been available for the determination of sodium. Barber and Kolthoff, in a recent paper(1), claim to have evolved a gravimetric method by which the rapid determination of this element can be made. It depends upon the fact that, when a solution of zinc uranyl acetate in acetic acid is added to an aqueous solution of sodium salt, a precipitate of the triple salt, zinc sodium uranyl acetate, having the composition $(UO_2)_3 . Zn . Na (CH_3 . COO)_9 . 6H_2O$ (containing 1·495 per cent. Na), is formed. The method of preparation of the reagent and the proportion of reagent to solution, time of standing, method of filtering, washing, etc., are described in the original paper by the aforementioned authors. They also show that cations such as Ca, K, and NH_4 may be present in the solution in considerable amounts without causing interference with the precipitation of the sodium. They consider that other cations will behave in a similar manner. It is pointed out that phosphate and large amounts of organic acids, such as oxalic and tartaric, may have an interfering action. From the composition of the precipitate, it will be seen that very small amounts of sodium may be estimated by this method, which suggests the possibility of adopting it for the determination of sodium in soil extracts. Bray(2) has successfully used the method for estimating the exchangeable sodium in soil leachings obtained with ammonium acetate solution. After certain preliminary treatments to obtain his solution for precipitation, he follows the technique described by Barber and Kolthoff, but recommends that the precipitate be allowed to stand for two days before filtration.

The present writer has shown(3) that the exchangeable bases of soil can be extracted by a semi-normal solution of acetic acid. The amounts of bases Ca, Mg, and K extracted by this reagent and normal ammonium chloride were found to show good agreement. A comparison of the amounts of extractable sodium was not made, because this exchangeable base was present in the soils under examination only in very small quantities, and, as it would have to be determined by difference, the determination would be loaded with the errors of the other determinations. In view of the agreement between the results with the two leaching

agents for the other exchangeable bases, it was assumed that a similar result would be obtained for sodium.

The development of the method of Barber and Kolthoff affords an opportunity of testing this assumption, and it suggests also that acetic acid would be the most convenient leaching agent for the estimation of exchangeable sodium, as the excess of leaching agent can be easily removed by simple evaporation without having recourse to ignition as in the case of ammonium acetate. In order to have a fair amount of sodium for estimation, the comparison was made on soils known to contain high proportions of sodium. The leaching was carried out in the usual manner with semi-normal acetic acid and normal ammonium chloride solutions. In the case of the acetic acid leachings 500 c.c. were evaporated to dryness with a few c.c. of strong hydrochloric acid. The residue was treated with a little aqua regia, evaporated to dryness and ignited over a rose flame for about one hour, extracted with water, filtered, and the filtrate made up to a known volume. The NH_4Cl extracts were treated with strong nitric acid, boiled to decompose the NH_4Cl, evaporated to dryness, ignited and treated in a similar manner to the acetic extracts. An aliquot portion (containing not more than 8 mg. of Na) was now evaporated to 1 c.c., and 10 c.c. of the zinc uranyl acetate reagent added, allowed to stand for one or two days, then collected, washed, and weighed. Corrections for blanks were made in every case. A preliminary experiment with a solution of sodium chloride gave the following results.

Time of standing after precipitation	Weight of precipitate gram	Weight of sodium chloride gram		Recovery (%)
		Found	Theoretical	
24 hours	0·495	0·01882	0·01905	98·81
	0·491	0·01867	0·01905	98·00
48 hours	0·494	0·01879	0·01905	98·65
	0·4935	0·01877	0·01905	98·54

In the following table, figures are given comparing the amount of sodium extracted from soils by the two leaching agents.

Table I.

Soil	Na_2O (%)	
	0·5 *N* HAc	*N* NH_4Cl
California A	0·197	0·217
California B	0·818	0·800
Russian alkali	0·373	0·357
Aber salt marsh (1)	0·425	0·431
Aber salt marsh (2)	0·482	0·493

The above figures agree sufficiently well to suggest that the category of sodium removed from the soil is the same by both leaching agents.

It may be remarked that the results of the blank determinations on the reagents were very much higher when ammonium chloride was used, and the corrections to be applied may be actually greater than the amount of sodium estimated in the case of non-saline soils when using this reagent for leaching. It is likely that most of this sodium comes from the nitric acid used to remove the excess ammonium chloride. A comparison of the two methods of leaching for such soils would not, therefore, be of great significance, and it may be claimed that the acetic acid extraction is more trustworthy.

In Table II some figures are given for the exchangeable sodium oxide of some representative Welsh soils.

Table II.

Soil	Type	Exchangeable Na_2O (%)
G 138 A	Lias. Heavy loam	0·0106
G 134 A	Keuper marl. Heavy loam	0·0058
G 232	Carboniferous drift. Heavy loam	0·0077
Ca	Palaeozoic drift. Medium loam	0·0071
G 233	Carboniferous. Light loam	0·0077
Merioneth	Palaeozoic drift. Medium loam	0·0087
Carm. 9	Alluvial. Silt loam	0·0091
A 76	Anglesey. Medium loam	0·0118
Mont. 5	Palaeozoic shale. Medium to heavy loam	0·0053
M 24	Old red sandstone. Light loam	0·0043
M 30	Alluvial. Clay loam	0·0084
M 18	Old red sandstone. Drift. Gravelly light loam	0·0045

The above figures indicate that in non-saline Welsh soils the percentage of exchangeable sodium is very low, and that it only forms a very small part of the total exchangeable bases. The results given in both tables include the water soluble and the exchangeable sodium. The writer found that, in some cases, small amounts of insoluble material, presumably silica, remained on adding water to the residue after evaporation prior to adding the precipitating reagent. The amount was so small as to be negligible in comparison with the weight of precipitate. After repeated extractions with water, filtration, evaporation and light ignition, duplicate solutions showed not more than 2 mg. difference in the weight of precipitate from those obtained after one extraction with water.

PROCEDURE.

(1) *Preparation of reagents.*

(*a*) Uranyl acetate ($2H_2O$) 10 gm.
Acetic acid (30 per cent.) 6 ,,
Water to make 65 ,,

(*b*) Zinc acetate ($3H_2O$) 30 gm.
Acetic acid (30 per cent.) 3 ,,
Water to make 65 ,,

(*a*) and (*b*) are dissolved by warming, the solutions mixed, allowed to stand for 24 hours and filtered. If the filtered reagent is kept in a Pyrex glass vessel, it does not become turbid on long standing.

(2) *Experimental.*

Leach out 25 g. of soil with 1000 c.c. of 0·5 *N* acetic acid. Evaporate 500 c.c. of this extract to dryness with a little strong hydrochloric acid. Add 1 or 2 c.c. of aqua regia and evaporate to dryness. Ignite over a strong rose flame for about 2 hours. Take up with water and filter. If only a small amount of sodium is suspected to be present, evaporate to dryness on a water bath. If a large amount of sodium is present, make up the filtrate to a known volume and estimate on an aliquot portion containing not more than 8 mg. of Na, if 10 c.c. of the zinc uranyl reagent is to be used. Dissolve in 1 c.c. of water and add 10 c.c. of the reagent, or in 2 c.c. of water adding 20 c.c. of the reagent, and allow to stand for two days. Filter through a dried, weighed asbestos Gooch crucible. Wash the precipitate five to ten times with 2 c.c. portions of the reagent, allowing each addition to run through before adding the next. Remove excess of the reagent by washing five times with 2 c.c. portions of 95 per cent. alcohol saturated with zinc sodium uranyl acetate. Finally wash out the alcohol with ether. The amount of ether used is immaterial, as the precipitate is insoluble in it. Draw air through the crucible to remove excess ether. Then place inside a balance case for at least 10 minutes before weighing. The weight of the precipitate multiplied by 0·01495 gives the weight of sodium.

SUMMARY.

1. The application of the zinc uranyl acetate method to the determination of exchangeable sodium in soils is discussed.

2. Using this method of determination, good agreement was found between the exchangeable sodium extracted by 0·5 *N* acetic acid and that extracted by *N* ammonium chloride.

3. The procedure for estimating sodium in acetic acid extracts is described, and the results for a number of typical non-saline Welsh soils are given.

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(*Received March 3rd*, 1930.)

SOME OBSERVATIONS ON THE SECONDARY SEX RATIO IN A GROUP OF DAIRY SHORTHORN AND WELSH BLACK CATTLE.

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The heavy wastage occurring in many dairy herds adds importance to the question of sex ratio in cattle, and it becomes an important factor in herds where it is desired to use only home bred cattle, as in herds free from tuberculosis and contagious abortion. The information required for the present investigation was obtained from Private Herd Books, particulars being taken as to sex of calf, month of birth and whether or not it was a first calf. Herds ravaged by contagious abortion were excluded, and also others if it was considered that there might have been neglect in entering births in the private herd book.

Table I.

Herd		Bull calves	Cow calves	Males per 100 females	Period covered
A*	Dairy Shorthorn	166	195	85·1	1912–April 1929
F*	Welsh Black	158	137	115·3	1912–April 1929
B	Dairy Shorthorn	359	378	95·0	1917–July 1929
C	,,	235	239	98·3	1910–May 1929
D	,,	145	152	95·4	1918–Dec. 1929
E	,,	123	95	129·5	1921–Dec. 1929
G	Welsh Black	397	427	93·0	1908–May 1929
H	,,	61	59	103·4	1918–May 1929
I	,,	115	98	117·4	1915–April 1929
J	,,	143	143	100·0	1910–April 1929
K	,,	54	72	76·3	1919–May 1929
L	,,	73	86	83·7	1910–April 1929
M	,,	32	23	139·1	1916–April 1929
N	,,	27	23	117·3	1926–June 1929
O	,,	31	33	93·9	1920–May 1929
P	,,	150	126	119·0	1910–July 1929
Q	,,	72	81	89·0	1924–Dec. 1929
R	,,	106	98	108·2	1909–July 1929
	Total	2447	2465	99·3	

* Herds A and F were at the same farm and under identical management.

Investigations into mammalian sex ratio have been mostly confined to the human race, owing to the mass of carefully prepared statistics available at certain sources, or to the polyembryonic and quickly maturing species, which yield a considerable amount of data in a com-

paratively short time. The sex ratio in cattle has not been frequently investigated. Crewe(1) refers to Wilckens as having obtained the ratio of 107·3 males per 100 females; Roberts and Yapp(2), with particulars of 8196 cattle, found the sex ratio to vary from 80·6 in Guernseys to 106·1 in Jerseys. In the present enquiry, comprising eighteen herds of Dairy Shorthorn and Welsh Black Cattle, the result is as follows:

Bull calves	Cow calves	Sex ratio
2447	2465	99·3

This ratio is nearer equality than those noted above. The results are given in greater detail in Table I.

Effect of breed.

Mussehl(3) found no significant differences between the sex ratios of three breeds of poultry. Roberts and Yapp(2) obtained the following ratios for five breeds of cattle: Holsteins 94·6, Ayrshires 96·0, Guernseys 80·6, Jerseys 106·1, and Herefords 101·8. The following results were obtained for the groups in this investigation:

	Bull calves	Cow calves	Sex ratio
Dairy Shorthorn	1028	1059	97·1
Welsh Black	1419	1406	100·9

The large fluctuation in the sex ratio from herd to herd would render it necessary to include a much larger number of herds to arrive at a figure which could be accepted as truly characteristic of the breeds as a whole. If, for example, this investigation had been brought to a close before Herd E, the last herd to be visited, had been included, a ratio of 93·9 would have been obtained for the Shorthorns instead of 97·1.

This consideration makes it impossible to attach much importance to results on single farms, but the striking difference on the farm where herds A (Shorthorn) and F (Welsh Black) are kept may be noted. This is the farm of the University College of North Wales, and great care has always been taken to keep the management of the two herds of cattle identical. The results throughout the 17 years have been most consistent. No difficulty has been experienced in maintaining the numbers of the Shorthorn herd without purchasing cows or heifers, but, largely owing to the preponderance of bull calves in the Welsh Black herd, females have been purchased nearly every year.

VARIATION FROM HERD TO HERD.

The sex ratio shows considerable variation from herd to herd, the extremes being 76·3 and 139·1, both of which were obtained from herds of Welsh Black Cattle. In the former case the data covered a period of 10 years, and in the latter 13 years. It is interesting to note that, in Herd K, where there were only 76·3 males per 100 females, the problem of keeping the herd up to strength was found to be more acute than in any of the others, heifers having to be purchased for this purpose. In one herd, J, with a total of 286 calves over a period of 19 years, the ratio was 100·0.

These fluctuations from herd to herd are most probably due to chance, but the figures should be examined for the possibility of some herds having a tendency to have a sex ratio peculiar to themselves, and differing from that of the group as a whole. King(4), for example, working with mice, succeeded in separating two distinct strains showing radically different ratios. An application of the "Goodness of Fit" test to the data contained in the table shows that, as far as these groups are concerned, the fluctuations in sex ratio from herd to herd are the result of chance. The method used for this test is one described by Fisher(5), and the table of χ^2 used is that contained in his work. Using the information set out above in the table giving the number of males and females in each herd, it can be calculated that $\chi^2 = 18{\cdot}86$. With n (degrees of freedom) $= 17$, the value of P, the proportion of cases in which any value of χ^2 will be exceeded, lies between 0·5 and 0·3, there being thus no evidence that the fluctuations are not due to chance.

EFFECT OF SEASON.

There appears to be ample evidence of seasonal variation in the mammalian secondary sex ratio. In the results of several investigators summarised by Crew(6), the sex ratio of dogs, pigs, sheep and cattle is lower in the winter months, that of 4900 cattle investigated by Wilckens being 103·0 for the colder and 114·1 for the warmer months. Parkes(7), from data of the albino mouse, and King and Stotsenburg(8) with the rat, had results supporting the contention that, in the season of highest birth-rate, the lowest proportion of males is obtained. King(9), in 1927, found a greater number of conceptions for mice, pigs and dogs in the first half of the year, with a high sex ratio for conceptions in the second half.

In this investigation, the ratio in the months March–August inclusive is compared to that in the other six months. This division has the

advantage that the first period in this case is the period of highest birth-rate, and also that it corresponds to conceptions beginning at the period of the spring flush of grass. The sex ratio for these two periods is shown below:

Period of Year	Male	Female	Sex ratio
March–August	1406	1483	94·8
September–February	1041	982	106·0

Thus, in the season of higher birth-rate (corresponding approximately to conceptions in the season when grass was plentiful), a lower sex ratio was obtained, as in the case of some of the investigators mentioned above.

If the two breeds are regarded separately in this respect, the difference due to season is apparent in both breeds, though to a greater extent in the Welsh Black Cattle. Thus:

Breed	Period	Sex ratio	Period	Sex ratio
Welsh	March–August	94·5	September–February	109·0
Shorthorn	March–August	95·1	September–February	100·0

Effect of age.

Investigations into other species have shown that the sex ratio varies with age, though the evidence is sometimes conflicting. In the case of poultry, Jull(10) found a higher ratio for the first lot of eggs than for the last lot in the same year, the ratio diminishing as the season advanced, thus suggesting a higher ratio for first conceptions. Parkes(11) found a lower ratio for subsequent births in mice than for first births; also that the sex ratio of infants declined with the age of the mothers. Fetschner(12), however, in the groups investigated by him, found that in the human race the ratio of males increased with the age of the mothers and with successive births.

Taking together the groups of cattle of both breeds under investigation, the sex ratio amongst a total of 1077 first calves was 102 males per 100 females, and amongst other cows (totalling 3834) 98·4 males per 100 females. If the two breeds are considered separately, conflicting results are obtained, the ratio being higher for first calves amongst the Shorthorns and for older cows amongst the Welsh; the numbers are probably too small to admit of considering the two breeds separately in this respect.

This problem is complicated by other considerations. As cows get older there is a tendency for an increasing proportion of them to calve in spring; thus, in cases investigated by the writer(13), only 17·2 per cent.

of first calvers calved in March, April and May, but the proportion calving in these months rose to 47·9 per cent. in the case of cows calving for the sixth time. If, as has been shown in several investigations, the sex ratio tends to be different at the season of highest fertility, then a change in sex ratio of older cows may be not only due to age or successive births, but also to the fact that a greater proportion of them calve at a certain period.

I wish to make grateful acknowledgment to Prof. R. G. White, at whose suggestion this enquiry was commenced. My thanks are also due to the owners and managers of the various herds for their kindness in allowing me access to their Private Herd Books.

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(*Received March 8th*, 1930.)

STUDIES IN SOIL CULTIVATION.

V. ROTARY CULTIVATION.

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(With Four Text-figures.)

Introduction.

When steam power was first applied on an extensive scale to cultivation operations in the first half of the last century, its main purpose was to haul the traditional cultivation implements, which were built to a larger size for this form of power. Multi-furrow ploughs, deep subsoilers and powerful cultivators were evolved, and the designs rapidly settled down to the present-day form of the familiar steam cultivation tackle.

However, a number of people considered that full use was not being made of this new form of power. Its essential characteristic was that it supplied a rotary motion, and it appeared to them wasteful, and even incorrect, to convert this rotation to a translatory motion for hauling the variety of implements necessary to produce a seed-bed. The identical result should be obtained—in theory at any rate—in one operation instead of several, by a transverse horizontal shaft fitted with radial tines, and revolved by power from the same steam engine that drew it across the field. The rotating tines would comminute and thoroughly mix the soil, leaving a seed-bed behind them. This process is now generally known as rotary cultivation. It was very persuasively described by Hoskyns in his *Talpa, or The Chronicles of a Clay Farm*, one of the most delightful books in the literature of agriculture:

> Is it not astonishing, with such experiences as we have before us in England, that since the first introduction of Steam-power to the notice and assistance of mankind, nobody has ever yet attempted to apply it *in its own way* to the definite and simple work of cultivation? It is put to cut chaff, to make saw-dust, to granulate powder, to make pins' heads, to reduce all sorts of *coarse material into fine*—and all by wheels,—*circular motion*, and nothing else, for nothing else will it accept,—but nobody can persuade his mind to believe that by the self-same action, and no other, it can cut

[1] Eleven past and present members of the Rothamsted staff and four voluntary workers have been associated with these experiments during the period 1926–9: W. B. Haines, E. M. Crowther, J. H. Coutts, C. Heigham, G. H. Cashen, G. W. Scott Blair, R. K. Schofield, H. G. Miller, A. R. Clapham, E. H. Gregory, J. Wishart; K. T. Hartley, D. W. Baker, G. E. Blackman, M. A. Sabet.

up a seam of soil eight inches deep and five feet wide, and leave it behind granulated to as coarse or fine a texture as the nature of the seed or season may require, and inverted in its bed. It is not ploughing, it is not digging, it is not harrowing, raking, hoeing, rolling, scarifying, clod-crushing, scuffling, grubbing, ridging, casting, gathering, that we want: all these are the time-honoured, time-bothered *means* to a certain RESULT. That result is—a seed-bed: and a seed-bed is, simply described, a layer of soil from six to twelve inches in depth, rendered fine by comminution, and as far as possible inverted during the process.

Machines embodying this principle were tried but without much success. They were heavy and cumbersome and did not produce a satisfactory tilth. On the other hand, a fair measure of success attended certain machines designed to produce not a seed-bed but a rough cultivation analogous to that given by the ordinary cultivator. These implements were "diggers" rather than rotary cultivators, the action of the spuds fixed to the rotating tines being to break the soil into large lumps and more or less to invert them. A few machines of this type were in use on heavy land in Essex until recently.

It appeared, therefore, that this pioneering work on rotary cultivation failed for two main reasons: firstly, the weight of the cumbrous machine, and secondly, the inability of the tines to produce as satisfactory a tilth as that obtainable with the traditional implements.

The invention of the internal combustion engine removed the first of these objections: the modern type is of considerably less weight per horse power than the old steam engine. The second objection is naturally more serious. Recent work on soil has emphasised the complicated nature of soil tilth and its controlling factors. But there is no *a priori* reason to assume that we have reached finality, either in the design of implements or in our cultivation methods.

These considerations led to the decision to make comparisons of rotary and traditional cultivation over a series of years at Rothamsted under field conditions. Experiments were made during the years 1926–9 inclusive, and the results form the subject of the present paper.

DESCRIPTION OF ROTARY CULTIVATION MACHINE.

The machine used for the Rothamsted experiments is of a type that has already been introduced for cultivation work in orchards, market gardens and commercial glasshouses.

It consists essentially of a 5 H.P. engine mounted on and geared to the two land-wheels, with a transverse horizontal shaft at the back, alsc driven by the engine. This milling shaft carries twelve radial tines, six on each side of the central line of the machine, and disposed symmetrically

about the shaft. The machine is steered by a long pair of handles which also carry the controls.

There are two forward speeds, about 2 and $\frac{3}{4}$ miles per hour. The rotation speed of the milling shaft is about 150 R.P.M., so the extent of forward movement for one complete revolution of the miller is about 14 in. at the high speed and 5 in. at the low speed. During this unit of forward movement each of the tines makes a complete revolution down into and up out of the soil. Each tine point, which is at a distance of about 8·5 in. from the axis of the milling shaft, therefore traces out a cyclic curve, the form of which for the fastest and slowest speeds is shown in Fig. 1. The part of this curve which is executed in the soil depends on the depth of tillage, which is adjusted within the limits of 2 in. and 10 in. by a depth shoe underneath the miller gear-box. A scale of depth is also shown in Fig. 1. Reference to the curve for the fastest speed shows that, as far as any single tine is concerned, a certain amount of the soil is left unstirred: this is represented by the area between the two loops of the cyclic curve, and has a minimum length of $3\frac{1}{2}$ in. and a maximum length of 14 in., this latter being represented by the distance between the lowest points of successive loops. But, as the tines are arranged in pairs roughly at opposite ends of a given diameter, the unstirred area in the figure is effectively dealt with by the second tine; the cyclic loops for this latter fall midway between those shown in the figure. In actual practice the tines are "staggered" with respect to the common diameter, so that they do not revolve in the same vertical plane in order to give a more uniform distribution of the cultivating action over the width of the work. It is evident that, in this case, some of the soil is only indirectly stirred by the tines[1]: the tine point will break away a lump of soil, and this may be carried round with the tine and thrown upwards against the miller hood, or it may be forced to one side and come under the action of an adjacent tine. On the other hand, the soil in the direct path of each tine is undoubtedly well pulverised, since in addition to the tine point, the whole body of the tine itself exercises a disintegrating action on the soil. Normally, therefore, for soil not initially in good physical condition (in which case almost any form of cultivation will produce a good tilth), the tilth produced by rotary cultivation will be a mixture of coarse and fine particles. This is an important point, because it is often urged that rotary cultivation produces too fine a tilth. The results discussed below will show this is not necessarily the case. Naturally

[1] When broad scuffling tines are fitted for shallow cultivation a greater bulk of the soil is directly stirred.

when the slowest forward speed is used much more stirring of the soil is effected. This is brought out by comparing the two curves in Fig. 1.

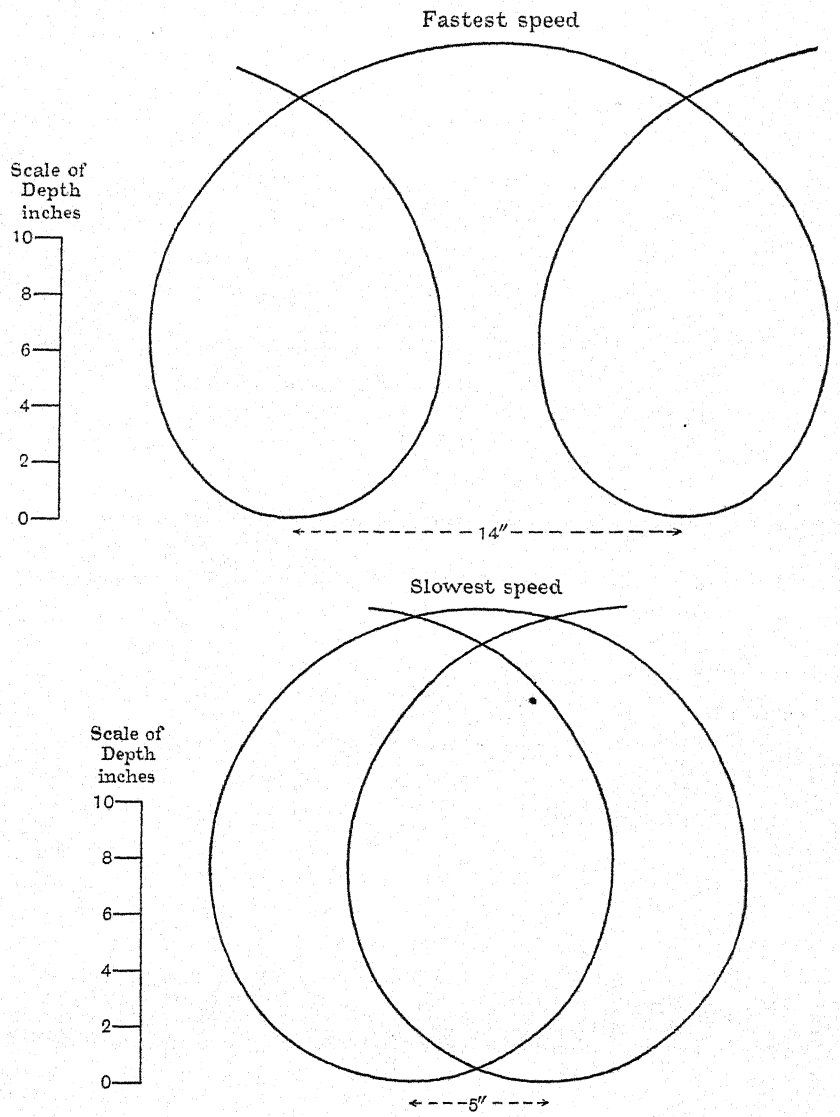


Fig. 1. Path of the tine points for fastest and slowest forward speeds.

Plan of the Rothamsted experiments.

The bulk of practical experience of rotary cultivation has so far been secured on light and sandy soils. The Rothamsted experiments provided a severer test owing to the nature of the soil. It is a heavy loam containing numerous flints, and demands some care in cultivation. For these reasons it was decided to confine the first series of experiments to spring cultivation and spring-sown crops only, and to defer the studies on autumn cultivations and autumn-sown crops to a later period. This second series of experiments is now in hand.

In all cases, therefore, unless stated to the contrary, the comparative experiments between rotary and ordinary cultivation were made on land that had been ploughed in the autumn or winter in the usual way. It will be realised that restriction of the experiments to spring cultivations necessarily postpones an answer to the question whether rotary cultivation is a satisfactory substitute for all our present cultivation operations, but it seemed advisable to attack the more difficult question of autumn cultivation only after some experience had been gained.

The observations fall into two main sections: measurement of the crop yield and records of germination and plant growth; measurement with a series of sieves of the amount of soil pulverisation or comminution produced by the given cultivation implement, and records of soil moisture and temperature at intervals during plant growth.

These two sections will be discussed separately below.

Section I. The crop results 1926–29.

The 1926 *experiments.* This was made on Sawyer's field, and was a comparison of three different methods of preparing a seed-bed for swedes. The preparatory "autumn" ploughing of 1925 was unavoidably delayed until February 1926. The land was wet and it was difficult to bury weeds. The land was left in the furrow until May 19th, when the triplicate plots received the following cultivations given on the stale furrows in the manner stated:

Series S. Rotary cultivated, rolled, drilled, rolled.

Series F. Tractor cultivated, harrowed, drilled, harrowed and rolled.

Series N. Ridged with bouting plough, rolled, drilled, rolled.

On each plot the depth of cultivation was 6 in.

As the object of the experiment was to study the effects of cultivation differences, it was considered advisable not to apply farmyard manure, which might have had the effect of masking these differences. A dressing

of fish meal, 4 cwt. p.a., superphosphate, 3 cwt. p.a., and muriate of potash, 1 cwt. p.a., was applied about a week before the experiment began, and a top-dressing of nitrate of soda, 1 cwt. p.a., was also given after singling.

In spite of the delay in winter ploughing and the weedy nature of the ground, the soil itself was in excellent condition, due no doubt to the severe frosts of the winter. Each of the three methods produced a satisfactory tilth. The rotary cultivation plots, however, were noticeably different from the other two treatments; the tilth was much looser and the foot sank some distance into it. This result is readily understandable. The rotating tines are travelling in an upward direction as they are leaving the soil. Not only is the tilth thoroughly loosened by this action, but a certain amount of the soil is actually thrown upwards, to fall more or less gently on the soil surface. It appeared that it would be difficult to use a horse drill on the soil in this condition, so a light rolling was first given to consolidate the soil.

After sowing, the plots were kept under observation. It was noteworthy that germination occurred earliest on the rotary cultivated plots, and this initial advantage was more than maintained in the early stages of growth. Shortly after germination the young plants were attacked by fly. The attack was not severe except on the ridged seed-bed plants, where a certain amount of re-seeding was necessary; the rotary cultivation plots escaped with little damage.

Frequent observation of the rate of growth was made after germination. Without exception the rotary cultivation plots were superior, the differences between the plants on these plots and the others being sufficiently great to be apparent to the naked eye. In view of this, and also because of the uncertainties due to the re-seeding of part of the Series N. plots, no detailed measurements of plant growth were taken.

All the plots suffered somewhat from weeds, and the available labour for horse hoeing, which was done at intervals during July and August, was insufficient to keep the weeds under. Docks and thistles were worst on the rotary cultivation plots, but this cannot be ascribed with certainty to the action of the rotating tines in breaking into smaller pieces the viable weed roots present at the time of cultivation. Although this effect undoubtedly occurred, it was also observed that many roots were pulled to the surface, where they died.

Singling was done unduly late in the season—again owing to labour difficulties and the large acreage of roots on the farm. To meet this situation the roots were first "bunched" by drawing across the rows a

cultivator frame carrying large ducksfoot tines grouped at suitable distances, so that hand labour was confined to singling the bunched roots. This operation left more roots on the rotary cultivation plots than on the remainder. In the first place, there were initially more gaps on the latter plots, owing partly to the inferior germination, and partly to the attack of fly already mentioned. In the second place, a number of the plants on the rotary plots either re-established themselves, or resisted the tines of the cultivator sufficiently to remain established in spite of injury. A similar observation was made on a closely adjoining area on which this method of bunching had to be employed (1), and a significant correlation (+ 0·671) was subsequently obtained between the ploughing draught (2) and the number of roots at harvest; the higher the draught the more resistant the soil and, therefore, the greater tendency both for the cultivator to ride out of its work and for partially uprooted plants to re-establish themselves.

This effect in the present investigation was closely associated with a field observation now to be described, which was both striking and unexpected. The loose tilth produced by the rotary cultivation and its effect on germination and plant growth have already been mentioned; it was observed that, in common with the other plots, the tilth was becoming more consolidated as time went on. This was of course to be expected; the Rothamsted soil tends to "cap" or to form a hard crust about 1 inch thick, and surface cultivation to break this crust is a standard procedure. On the rotary cultivation area, however, the effect was far more intense, and these plots could be picked out by the mere feel of the land underfoot; the hardening or capping effect had extended to a much greater depth in this case. The effect on the plants was equally striking. Before the capping they were obviously ahead of those on the other plots, but afterwards they made little further growth, and from being the best and most forward plants, they finished up the season as definitely the worst and least developed. The harvest yields are given in Table I.

The yields on the S. plots are significantly below either of the other two treatments, which do not differ significantly from each other. This difference is all the more striking in view of the significantly greater number of roots on the S. series of plots as shown in the table.

The negative factor responsible for the differences in root number was the severer losses due to attack of fly on the N. and F. series, only partly remedied by the re-seeding, while the positive factor was the survival of many roots on the S. series, owing to the ineffective method of "bunching" with the cultivator on the already hardened soil. A

measure of the size and weight distribution of the roots was obtained from some 230 sample roots from each plot. These were obtained by pacing consecutively up one row and down the next, and pulling the nearest root after a definite number of paces. The size distribution was measured on the basis of bulb diameter, using a set of ten gauges, each of which was nine-tenths the size of the preceding one. The gauges began at 17·3 cm. and did not extend below 7·8 cm. in diameter. All roots 7·8 cm. in diameter were put into the bottom class, which may be regarded as representing the number of useless roots for practical purposes. The results are shown in Fig. 2, which demonstrates clearly that the average size of the roots is lowest on the rotary cultivated plots and greatest on the ridged plots. The size distribution curve for each series also shows a considerable number of roots in class 0, *i.e.* of less diameter than 7·8 cm., especially on the rotary cultivation series, due to the survival of many plants from the bunching operation and the severe check to growth following the extensive hardening of the soil.

Table I. *Weight of roots per acre in tons, and number of roots per acre* (1926).

Plot No.	Rotary cultivation (S.)	Flat seed-bed (F.)	Ridged seed-bed (N.)
1	9·88	12·25	11·56
2	9·55	10·84	12·86
3	8·77	10·06	11·00
Average	9·40	11·05	11·81
Average percentage of general mean	87·5	102·8	109·8
Average no. of roots p.a.	15,784	13,157	10,693

Standard error of averages: 0·403 ton and 3·75%; 821·7.
Significant difference: 1·140 tons and 14·4 %; 2324.

The weight distribution for the sample roots was taken to the nearest ounce for each root, and in Fig. 3 the results are given in 5 oz. steps. As would be expected, these curves are more regular than those of Fig. 2, and they bring out very clearly the differences in growth with the three cultivation treatments.

The 1926 experiments, then, showed a distinct advantage for rotary cultivation in the germination of seed and the early stages of growth, followed by a remarkable reversal in the later stages due to extensive hardening of the tilth, resulting in an appreciable reduction of yield. This effect at once raised the question whether it was an inevitable accompaniment of this form of cultivation on heavy soil, and to what extent it could be prevented.

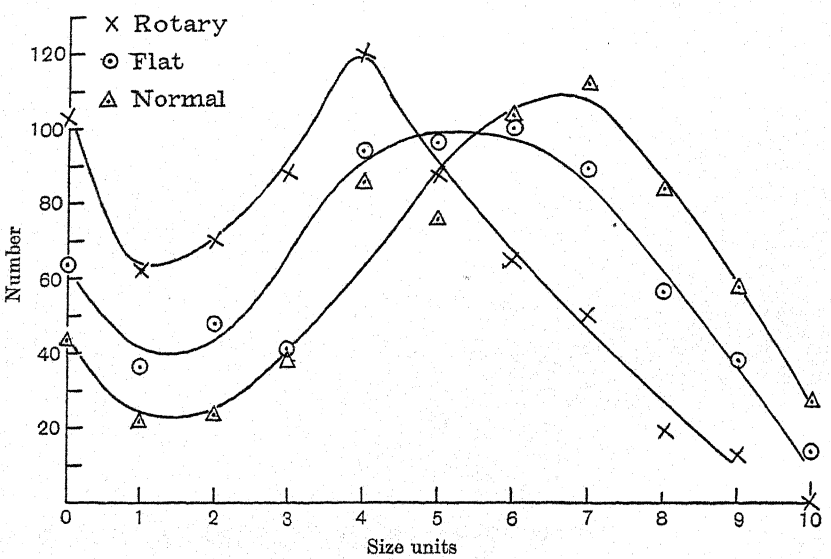


Fig. 2. Size distribution of roots, 1926 experiments.

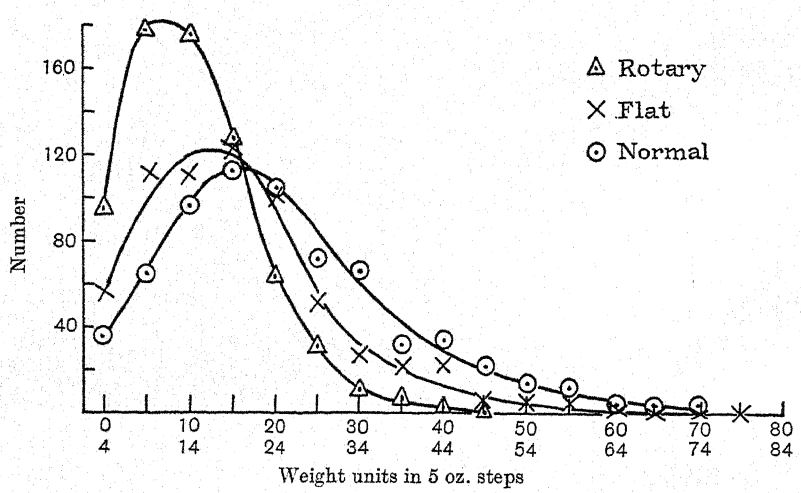


Fig. 3. Weight distribution of roots, 1926 experiments.

The 1927 experiments. It was decided to follow up the 1926 results by retaining the same plots, and to sow them to another spring crop. Barley was selected as the normal crop in the rotation. As the comparative experiments were to be confined to spring cultivations only, and in view of the very hard condition of the soil on the S. series, the whole area was cross-ploughed in January. Unfavourable weather prevented further operations until April.

It was proposed to repeat the rotary cultivation on the S. plots of 1926, to horse cultivate and disc harrow the F. series, and to plough (lengthwise), drag harrow and ring roll the N. series. Thus, as in 1926, rotary cultivation would be compared with two other standard traditional methods of spring cultivation. Owing to a mechanical defect, only one of the three rotary cultivation plots received this treatment; the remaining two were treated in the same manner as the F. series. The treatments in 1927 compared with those for 1926 are given below for convenience of reference.

All plots ploughed.

1926			
Spring treatment	Rotary cultivated, rolled, drilled rolled	Tractor cultivated, harrowed, drilled, harrowed, rolled	Ridged with bouting plough, rolled, drilled, rolled
Descriptive letter	S.	F.	N.

All plots cross-ploughed.

1927			
Spring treatment	(One plot only) rotary cultivated, harrowed, drilled, harrowed & rolled, (other two plots) treatment as in C.	Horse cultivated, disc harrowed, harrowed, drilled, harrowed & rolled	Ploughed lengthwise, drag harrowed (twice), ring rolled, harrowed, drilled, harrowed & rolled
Descriptive letter	S.′ for single plot. C.′ for other two.	C.	P.

The operations were spread over the period April 5th–May 9th, and included manuring as follows: superphosphate, 3 cwt. p.a., muriate of potash, 1 cwt. p.a., and sulphate of ammonia, 1 cwt. p.a. The wet weather in the early part of the year and a dry spell previous to the spring cultivations produced an unkindly condition in the soil, and made it difficult to obtain a tilth. In consequence, sowing was delayed and the harvest was correspondingly late.

Observations of the plots were made at intervals, and showed unmistakably that growth on the old S. series of 1926 (*i.e.* the S.′ plot and the two C.′ plots in the present series) was much better than on any of the other plots. There was no sign on the one rotary cultivated plot (S.′) of

the remarkable effect obtained with swedes in 1926. The superiority of the S.′ and C.′ plots over the remaining six was easily visible to the naked eye, and was carried on into the harvest yields both of grain and straw. The mean results are shown in Table II.

Table II. *Yields per acre.* (*Barley* 1927.)

Plots	S.′ & C.′	C.	P.
Grain (bushels)	27·8	21·6	21·9
Percentage of general mean	117·0	91·0	92·0
Standard error 1·16 bush.	Significant difference 3·28 bush.		
Straw (cwt.)	21·7	18·6	18·3
Percentage of general mean	111·1	95·3	93·6
Standard error 0·46 cwt.	Significant difference 1·30 cwt.		

This table shows that the plots which, in 1926, gave yields significantly below the remainder, have in 1927 given yields significantly above the others. As there was no appreciable difference in yield between the S.′ and the two C.′ plots in spite of the difference in cultivation, the effect must be ascribed to some residual action from the preceding year. There is the obvious possibility that more plant food was available for the barley on the old rotary cultivation plots owing to the lower yield of swedes given in the preceding year. But a simple manurial residual effect of this nature seems very unlikely in view of the small amounts of nutrients involved. There was a deficiency of approximately 2 tons of swedes on the S. series of 1926, and an excess of approximately 6 bushels of barley, and the corresponding amount of straw, on the same plots in 1927. Taking accepted figures for the N, P and K contents of these crops, we obtain:

	Lb. per acre.		
Crop	Nitrogen	Potash	Phosphoric acid
Swedes (2 tons)	10·0	9·0	2·4
Barley (6 bushels & straw)	7·2	5·4	3·1

There is admittedly an approximate equality in the two sets of figures, but the amounts are small in comparison with the manures that were given to the barley crop; further, a proportion of the nutrients would have been washed out from the soil during the winter. The effect is by no means easy to understand, and, as something very similar also appeared in the later experiments of 1927 and 1928, described below, it appears to be a probable accompaniment of rotary cultivation.

The 1928 *experiments.* These experiments were transferred from Sawyer's field to Great Harpenden field, to allow of a more extended series of plots.

A composite soil sample was taken for mechanical analysis from the experimental area of each field. The figures show that the areas were practically identical in mechanical composition, and direct comparison of the experimental results is therefore legitimate.

Mechanical analysis (*Revised Official Method A.E.A.* (3)).

	Coarse sand	Fine sand	Salt	Clay	Air dry moisture	Loss by solution	$CaCO_3$	Difference	Total
Sawyers	12·8	40·2	23·0	19·5	2·2	0·7	—	1·6	100·0
Gt. Harpenden	7·8	41·3	23·3	22·3	2·6	0·8	—	1·9	100·0

Swedes were selected for the crop, and the experiment included trials of possible methods of preventing the hardening of the soil noted with rotary cultivation in 1926, with the idea that the superiority in the early stages of growth might then be maintained over the whole growth period.

The land was winter ploughed in December 1927, and left in the furrow until May 4th, when a thistle cutter was used to kill surface weeds, which were fairly numerous. On May 5th the following manure was sown: sulphate of ammonia 2 cwt., superphosphate 2 cwt., and muriate of potash, 2 cwt. (all per acre).[1]

The following four cultivation treatments, each in quadruplicate, were given over the period May 7th–9th:

Series A. Horse ploughed, harrowed (twice) and ridged.

Series B. Rotary cultivation followed by ridging.

Series C. Rotary cultivation (left flat).

Series D. Rotary cultivation (left flat) to be followed by a second shallow rotary cultivation at a later date.

Series A. and C. were a repetition of the 1926 trials; Series B. and D. were intended to compare two possible methods of overcoming the hardening of the soil. Method D. was based on the idea that if the surface "cap" were broken at an early stage it might not extend downwards; method B. embodied the assumption that the "cap" would be less likely to form on the sloping surface of the ridges than on the level soil.[2]

[1] A portion at one end of the experimental area, covering nearly the whole of the first block, inadvertently received a dressing of dung which was being applied over the rest of the field. Statistical examination showed that the response to the cultivation treatments was very much the same on the dunged and undunged areas, while the extra variation due to the higher yield of the dunged plots, being mostly a block effect, was eliminated from the comparisons. The yield data given in Table III were not, therefore, corrected for this effect.

[2] The ridging would normally be done at the same time as the rotary cultivation, by a ridging attachment fitted behind the rotating shaft. In this experiment suitable ridging breasts were not available, and an ordinary bouting plough (horse drawn) was used.

Seed was sown on May 9th, and the whole area was then rolled. Germination was again earliest on the rotary cultivation plots, but there were no marked differences in subsequent growth. Singling was done on June 18th, and was immediately preceded by hoeing on all the plots. A second horse hoeing was given on July 3rd, and a final hand hoeing on July 12th. Although these hoeings interfered with the original scheme, in that they would probably tend to prevent the soil-hardening effect which treatments B. and D. were designed to study, it was judged essential that they should be carried out in view of the weed infestation. Careful watch kept on the whole area from the date of germination disclosed no signs of capping, or of differences in growth between the various cultivation treatments. It was reasonable to conclude either that the capping effect was absent altogether, or that any tendency for it to occur could be defeated by a light cultivation in its early stages. At the time of the last hoeing on July 12th, the plants had made considerable growth; it was evident that the second light rotary cultivation arranged in treatment D. would involve some danger of damage to the leaves of the plants from the rotating tines. Although there was still no sign of capping, it was decided to give the second rotary cultivation to meet the possible contingency of the effect occurring later in the season. The operation was done on July 20th, and a certain amount of damage to the leaves of the plant was unavoidable.

The roots were counted, lifted, and weighed during the period November 21st–25th, and a series of girth measurements were taken on sample roots from each treatment. The yields are summarised in Table III, and the frequency curves for growth measurements are shown in Fig. 4.

Table III. *Yields and root numbers.* (*Swedes*, 1928.)

Series	A.	B.	C.	D.		
Cultivation treatment	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation	Means	Standard errors
Roots:						
Tons per acre	22·67	22·80	20·12	18·02	20·90	0·50
Percentage	108·5	109·1	96·2	86·2	100·0	2·39
Tops:						
Cwt. per acre	21·03	20·92	17·08	16·05	18·77	1·13
Percentage	112·0	111·4	91·0	85·5	100·0	6·03
Roots:						
No. per acre	19,560	19,390	16,620	15,470	17,760	338·8
Percentage	110·1	109·2	93·6	87·1	100·0	1·9

Treatments C. and D. have resulted in a significant depression in yield both for roots and tops; further, treatment D. is significantly below C. in root yield.

We thus have a repetition of the results obtained in 1926, although the depression in yield with comparable treatments (C. in Table III, S. in Table I) is not so large. There is the further result that the second shallow rotary cultivation in Series D. was associated with additional drop in the yield of roots. The immediate explanation of these results would be to ascribe the reduction in yield to rotary cultivation, but a fuller examination puts the matter in a different light. In the first place, treatment B.

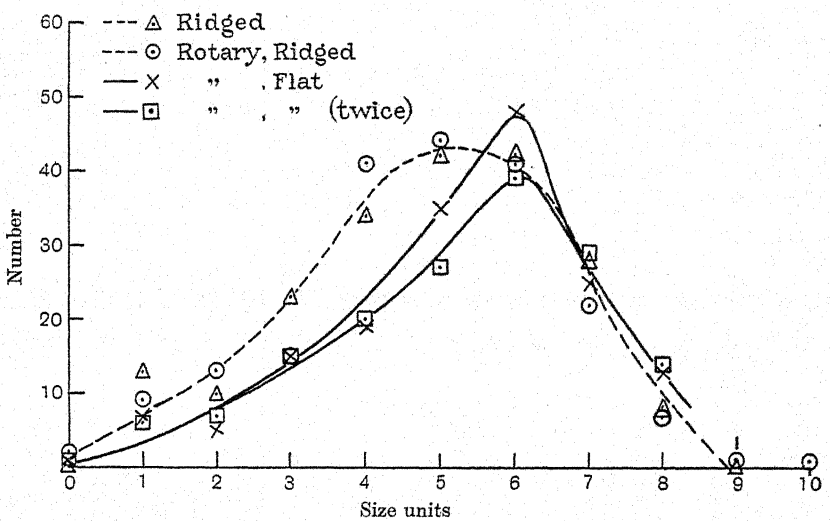


Fig. 4. Rotary cultivation 1928 experiments. Size distribution of roots.

has given yields equal to the standard treatment A., although it included rotary cultivation. In the second place the reduction in 1926 was definitely associated with a capping or hardening of the soil, and there were no signs of this in 1928. In the third place, an examination of Table III shows that the yield in each of the four treatments is closely proportional to the actual number of roots on the plot. The closeness of this agreement is well shown by comparing the second and last lines in the table. The differences in yields may therefore be ascribed to differences in number of roots, more especially as Fig. 4 shows that the size distribution of the roots was much more uniform over the different cultivation treatments than was the case in 1926 (Fig. 2). A number of possible causes suggest themselves for this decrease in the number of roots but,

in the absence of the necessary data, no definite explanation can be advanced.

The 1929 *experiments.* The similarity between the 1926 and 1928 results made it highly desirable that the residual effect of 1927 should again be looked for, and it was therefore decided to follow the general plan of the 1927 experiments. The weather prevented winter ploughing, and the whole area was ploughed, using horse teams, early in March. The land was then tooth harrowed immediately before and after drilling with barley on March 14th. Manures were sown on March 27th and consisted of 2 cwt. super-phosphate, 1 cwt. muriate of potash and 1 cwt. sulphate of ammonia, per acre. This set of plots will be referred to below as the Old Set.

At the same time a second set of 16 plots (New Set) was established only a few yards from the Old Set, on land that had also carried swedes in 1928. This area had also received 16 tons per acre of dung as well as artificials, and was therefore in a better state of fertility than the Old Set plots. The purpose of the New Set was to test rotary cultivation as a seed-bed preparation for barley. The land had had no winter ploughing after the swedes were removed. The comparison was, therefore, the direct one between spring ploughing and harrowing, and rotary cultivation, and there were eight plots for each of the two cultivation treatments. The operations were carried out, and the land was manured and sown at the same time as the Old Series. The season was an excellent one, and good growth and yields were obtained both on the Old and New Sets.

The results for the Old Set are summarised in Table IV.

Table IV. *Old Set.* (*Barley*, 1929—*all plots ploughed and harrowed.*)

Series	A.	B.	C.	D.		
Cultivation treatment in 1928	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation	Mean	Standard errors
Grain:						
Cwt. per acre	27·2	27·3	24·8	28·4	26·9	1·11
Percentage	101·1	101·2	92·3	105·5	100·0	4·13
Straw:						
Cwt. per acre	35·0	37·3	33·5	41·2	36·7	1·72
Percentage	95·2	101·4	91·3	112·1	110·0	4·67

There is no significant difference between the old rotary cultivation and ordinary cultivation plots, as far as the yield of grain alone is concerned, and the striking observation of 1927 (Table II) is therefore

not repeated. For the straw the differences just approach the level of significance. It is interesting to note that, while series C. is still below the mean, as in 1928, series D. gives the highest yield and appreciably exceeds A. and B. In 1928, treatment D. was significantly below C., which in turn was below A. and B. We thus have a partial repetition of the 1927 results, in that an area which gave under a rotary cultivation treatment the lowest yield of swedes, gives under ordinary cultivation in the succeeding year the highest yield of barley straw and grain.

In the New Set, counts were made of the number of plants present about one month after sowing. The usual significant difference in favour of the rotary cultivation was observed. It was also noticed that higher numbers of plants occurred in the centre of the plots than on the sides, in both the ploughed and rotary cultivated series. In the ploughed series this difference is understandable. In distinction to the 1928 experiments, where a one-way plough was used, the ploughed plots were ploughed in narrow lands in 1929, in the ordinary manner. In consequence the ridge with its deeper tilth ran down the middle of each ploughed plot and the furrows along each edge. This explanation will not serve for the rotary cultivated plots, as the work was begun at one side of the plot and proceeded regularly to the other side, and the depth of tilth was therefore sensibly uniform.

During the later stages of growth no differences could be seen, and the small differences in early growth recorded above were doubtless soon levelled by tillering of the plants. An excellent crop was obtained, and the yields are shown in Table V.

Table V. *New Set.* (*Barley*, 1929—*no autumn ploughing.*)

Series	E.	F.		
Cultivation treatment in spring	Ploughed and harrowed	Rotary cultivation left flat	Mean	Standard errors
Grain:				
Cwt. per acre	30·50	29·93	30·22	0·575
Percentage	101·0	99·0	100·0	1·90
Straw:				
Cwt. per acre	44·93	43·75	44·34	1·192
Percentage	101·3	98·7	100·0	2·69

Table V shows that both rotary and ordinary cultivations gave excellent crops, there being no significant difference between them. The yields are appreciably higher than those shown in Table IV. The difference is attributable to the residual value of the dung given to this area in 1928.

Summary of the crop results, 1926–9.

Rotary cultivation as a means of seed-bed preparation for swedes and barley gives earlier and better germination of seed, and superior early growth. This initial superiority, however, is not maintained. In the case of swedes actually a lower yield is given, which was definitely associated with a hardening of the soil during the growth period in the 1926 experiments, but not in the 1928 results. Rotary cultivation is satisfactory for barley, and yields are obtained equal to those given with ordinary cultivation.

The reduction of yield of swedes due to rotary cultivation is followed by a marked increase in the yield of the succeeding crop (barley), when the plots in question are cultivated in the ordinary way.

Section II. Measurements of soil comminution.

This section describes the measurements made to ascertain whether there were any marked differences produced on the soil by the various cultivation implements used in the four years' experiments. The method selected was to measure the proportion of soil remaining on each one of a set of different sized sieves, samples of soil being taken for this purpose immediately before and after the passage of the implement. In any experiment a considerable number of samples is needed from each plot on account of soil heterogeneity.

Four sieves were used: three had square apertures of sides of 1·5 in., 0·5 in., and 0·25 in., while the fourth was the usual 3 mm. round hole type, and may with sufficient accuracy be taken as equivalent to a square meshed sieve of 0·1 in. side. The successive passage of a soil sample through this set of sieves gave five groups, (*a*) to (*e*), group (*a*) consisting of lumps not passing the 1·5 in. sieve, and group (*e*) of soil crumbs small enough to pass the 0·1 in. sieve. Samples were taken with a spade, a cube of soil being isolated by vertical cuts with as little disturbance as possible. The spade was then carefully inserted under the cube, which was transferred with the minimum disturbance to a sheet of stout paper and taken to the sieves. The sample was passed progressively through the set of sieves, a gentle oscillation of each sieve being maintained until no more soil passed through. Each group was then weighed and expressed as a percentage of the total weight of the sample. The weights are not corrected for the moisture in the soil. Measurements of the moisture content were always taken, and showed but little variation during any test. The comparisons of the sieving results before and after

cultivation are not, of course, affected by including the moisture content. Further, the average moisture values in the successive years are not sufficiently different from each other to invalidate direct comparisons of the sieving results from one year's experiments to another. The stony nature of the land presented a difficulty, especially in groups (*a*) and (*b*), which sometimes contained an appreciable proportion of stones. It was decided to remove by hand from these groups all stones larger than about $\frac{1}{2}$ in. in diameter before weighing the sample. In the tables below, besides the percentages of the five fractions, there is given a "surface area" figure. This is a rough measure of the total surface area of the individual pieces making up the whole sample, and serves as a "single value" measurement for specifying the amount of comminution in any sample. To obtain this figure it was assumed that the pieces were cubes, and that the average size of these lumps of soil on any sieve was midway between this sieve aperture and the one immediately above. The sides of the cubes were therefore approximately 0·05 in., 0·2 in., 0·4 in., 1 in., while for the fraction on the largest sieve (whose contribution to the total area is small in any case) a value of 2 in. was arbitrarily assigned. The surface area of any group is then proportional to the number of pieces multiplied by A^2, where A is the length of the cube edge; the number of pieces is in turn proportional to the percentage of that group divided by A^3. Hence, the surface area is proportional to the percentage of that group divided by A.

The 1926 *measurements.* As already mentioned in Section I, the soil was in excellent condition, and was ready to fall down into a good tilth under almost any form of cultivation. In fact, sampling before the cultivations had to be done with considerable care, otherwise the soil block fell to pieces in the process. The percentages of the various groups isolated by the sieves are given, together with the surface figure, in Table VI.

Table VI. *Seed-bed for swedes,* 1926.

	Before cultivation (ninefold replicates), percentage of each group			After cultivation (ninefold replicates), percentage of each group		
Group	Rotary S.	Flat F.	Ridging N.	Rotary S.	Flat F.	Ridging N.
(*a*)	10·9	7·5	14·1	1·9	4·3	6·0
(*b*)	27·9	32·9	27·0	21·8	24·6	20·8
(*c*)	17·7	19·6	18·8	17·7	18·8	15·6
(*d*)	24·4	13·2	23·5	27·6	26·7	26·2
(*e*)	19·1	16·8	16·6	31·1	25·6	31·5
"Surface"	581	538	531	827	719	824
Percentage increase in surface after cultivation :—				42	34	55

Inspection of the before-cultivation results shows a reasonably close agreement in the proportions of the different groups present, indicating that the ninefold replicates taken (three on each of the triplicate plots) are adequate. The surface figures do not agree so closely, since they are sensitive to small changes in the amount of fractions (*d*) and (*e*). Comparison of the group percentages before and after cultivation shows that, within the limits of the sieving method, there is little to choose between the three methods of cultivation in efficiency of disintegration of the soil. However, the surface figure and the corresponding percentage increases offer some suggestion that ridging was most effective, followed by rotary cultivation and flat seed-bed treatment, in the order named. This sequence, it will be found, is repeated in the later experiments.

Table VI shows that rotary cultivation did not produce a much finer tilth than traditional methods, even in this soil which was peculiarly ready to disintegrate. It would, therefore, appear that the frequently made assertion that rotary cultivation produces too fine a tilth is incorrect. It certainly produces a much looser tilth, for reasons already given, and probably this appearance of the soil after cultivation has led the practical man to interpret it wrongly as a finer tilth.

Frequent measurements of moisture content were made during the season, and a recording thermometer was installed at the 6 in. depth on each of the three plots in the central block, further temperature records being taken on selected occasions at different depths with ordinary mercury thermometers. Examination of the results fails to show any close connection between them and the observed behaviour of the crop, with the exception of the temperature records for the first weeks after sowing. The rotary cultivation plot gave higher daily minima and maxima than either of the other plots, and the earlier germination is doubtless due to this.

The remarkable slowing up in growth already discussed must be attributed, therefore, to the consolidation of the soil, and not to any extra fineness in the tilth, or to moisture and temperature factors.

No further measurements were made on the plots in 1927.

The 1928 *measurements.* These results form a useful contrast to those of 1926. They refer to a different field which, however, is practically identical in mechanical composition to the original one. At the time of the cultivations, the land was still suffering from the wet season of 1927 and the winter of 1927–8, and its physical condition was distinctly inferior to Sawyer's field in 1926. The difference was easily apparent

during the actual cultivations and is well brought out by the sieving tests, recorded in Table VII.

Table VII. *Seed-bed for swedes*, 1928.

Before cultivation (twelvefold replicates): percentage of each group.

Series	A.	B.	C.	D.
Cultivation treatment	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation
Group				
(*a*)	60·2	61·8	58·2	60·8
(*b*)	11·3	11·2	13·9	12·5
(*c*)	11·1	10·4	11·2	11·4
(*d*)	8·0	7·4	7·8	6·7
(*e*)	9·3	9·3	8·8	8·5
"Surface"	295	291	286	274

After cultivation (twelvefold replicates): percentage of each group

Series	A.	B.	C.	D.
Cultivation treatment	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation
Group				
(*a*)	28·9	14·8	48·5	42·3
(*b*)	17·7	20·1	12·3	13·8
(*c*)	20·2	23·6	14·4	14·7
(*d*)	13·3	18·3	11·0	11·8
(*e*)	19·8	23·2	13·8	17·3
"Surface"	546	640	403	477
Percentage increase in surface after cultivation :—	85	120	41	74
			58	

Comparison of the before-cultivation figures with those of Table VI for 1926 shows the physical difference that existed between the two soils. The outstanding feature is the large percentage of group (*a*) in 1928, and the low values for the two finest groups (*d*) and (*e*). A comparison of the after-cultivation results 1928, with the before-cultivation figures 1926, is even more striking. Except for group (*a*) in series C. and D. the general run of the figures in the two years, including the surface values, is much the same. It appears, therefore, that in 1926 the gentle disturbance incurred in taking the sample and sieving it gave the same final result as the drastic operations of rotary tillage, or of ridging, in 1928. The two tables clearly show the great influence of climatic conditions on the tilth of the soil.

Examination of the actual effects of the cultivation operations in 1928 also show important differences. Further evidence that rotary

cultivation does not necessarily produce a finer tilth than ordinary methods is given by the high figures for group (*a*) in series C. and D. in comparison with series A.

A further point of importance is the demonstration of the great value of a ridging or bouting implement in breaking up unkindly soil. It is at least as effective as rotary cultivation, while the combination of the two is naturally the most efficient of the three treatments. The net effect of the combination as judged by the percentage increase in the surface area figures is an additive one, although this conclusion is made less certain by the wide difference between series C. and D. (41 per cent. and 74 per cent. respectively). The difference is a little disturbing as treatments C. and D. were identical, the second rotary cultivation on D. being done at a later date. Further, the results are the average of twelvefold replicates in each case.

As already mentioned, there was no capping or hardening of the soil in 1928; the coarser tilth in this year, which is well brought out by the sieving results, is the probable reason for its absence.

The 1929 *measurements.* There were two separate experimental areas in this year. One set consisted of the 1928 area, which was ploughed and harrowed throughout, and sown to barley to see whether the reduced yield of swedes on the rotary cultivation plots would again be followed by an increased yield of barley. The other set was a direct comparison on adjacent land of rotary and ordinary cultivation as a seed-bed preparation for barley. The sieving results are shown in Tables VIII and IX respectively.

Table VIII. *Barley (Old Set)*, 1929.

(Cultivation treatments in 1928 are shown in Table VII.)

	Before ploughing.			
Series	A.	B.	C.	D.
Group				
(*a*)	25·1	36·1	35·4	29·3
(*b*)	22·1	19·0	18·3	22·3
(*c*)	10·1	9·0	9·1	9·8
(*d*)	28·3	23·4	24·9	24·6
(*e*)	14·4	12·5	12·3	14·0
"Surface"	490	426	429	464
	After ploughing.			
Group				
(*a*)	20·8	24·3	24·4	30·1
(*b*)	23·0	21·6	20·0	19·7
(*c*)	12·8	12·2	13·8	12·8
(*d*)	35·0	31·1	31·0	28·6
(*e*)	8·4	10·8	10·8	8·8
"Surface'	409	436	438	386

Table IX. *Barley* (*New Set*), 1929.

Series	E.	F.	E.	F.
Cultivation treatment	Ploughed and harrowed	Rotary cultivation	Ploughed and harrowed	Rotary cultivation
	Before cultivations		After cultivations	
Group				
(*a*)	37·6	36·7	29·5	13·2
(*b*)	21·7	22·4	14·6	17·1
(*c*)	9·5	9·6	10·5	13·2
(*d*)	24·7	22·9	32·5	43·6
(*e*)	6·5	8·4	12·9	12·9
"Surface"	318	346	476	533
Percentage increase in surface after cultivation :—			50	54

The before-ploughing figures in Table VIII compared with the after-cultivation values for the same area in Table VII show that only a moderate amount of consolidation had taken place in the interval. The severe cold spell in February 1929 probably counteracted to some extent the general consolidation of the soil during the winter. The figures also show that any difference due to the different methods of cultivation in 1928 had been evened up by the time of the 1929 ploughing.

Table VIII also brings out the surprising result that ploughing did not, in the particular conditions of this experiment, produce any appreciable shattering effect on the soil, whether one takes the percentage distribution of the sieve fractions or the surface figures as the criterion. There is a suggestion that the plough produced a sub-division of the largest sized lumps, and at the same time an aggregation of the smallest lumps into larger ones, for group (*d*), particularly, is increased by ploughing and groups (*a*) and (*e*) are decreased. A decrease in group (*a*) would certainly be expected, no matter what physical state the soil was in, while the decrease of group (*e*) could be explained by the compressing effect of the tail-end of the mould board on the furrow slice as it is pressed into position. But when the before- and after-ploughing figures for the New Set of plots (Table IX) are compared with those just discussed, it is observed that, in this case, ploughing did have an effect, amounting to about 50 per cent. increase in the surface figure. This area had received a liberal supply of organic manure in 1928, and was hence in better tilth than the Old Set.

On the New Set of plots, the effect of rotary cultivation is seen to be little different from ploughing, except that the proportion of group (*a*) is much less. This result, which was not obtained in 1928, is probably also due to the better inherent tilth in 1929, following the application of organic manure in the previous year.

Summary of Section II.

The outstanding feature of the results is the predominant effect of meteorological factors on the physical condition of the soil. If the soil is in good condition, almost any form of cultivation will produce a satisfactory tilth.

The general effect of rotary cultivation is to leave a smaller proportion of large lumps and hence a more uniform tilth, but it does not produce a much finer tilth than the traditional forms of cultivation. It leaves a much looser or "puffed up" seed-bed, the appearance of which has no doubt given rise to the frequent but incorrect assertion that rotary cultivation produces too fine a tilth on loams or heavy soils.

The ridging or bouting plough is shown to be an effective implement for breaking up soil in poor physical condition, and is appreciably superior to the ordinary plough. A ridging body attached to a rotary cultivation implement is even more effective.

The ordinary plough does not produce much comminution of the soil unless it contains a good supply of humus, and is therefore in good physical condition. At other times the tendency is to increase the percentage of medium-sized lumps, at the expense of the largest and smallest, the latter becoming aggregated by pressure from the tail-end of the mould board as the furrow slice is pressed into position.

General discussion.

This section briefly supplements the detailed discussion given in Sections I and II.

The outstanding feature of the four years' experiments on rotary cultivation for preparing a seed-bed for spring crops is the contrast between the beginning and end of the growth period. Practically without exception, quicker and better germination, and more rapid early growth, have followed from this method of cultivation. But, again without exception, this initial advantage has either been lost or even replaced by a reduced yield at harvest. The absence of any significant difference in the yield of barley between the rotary cultivated and normally cultivated plots is not surprising; Eden and Maskell(1) have shown for wheat that a reduced germination percentage implies a greater available root range for the survivors, and is thus associated with more abundant tillering, which in consequence levels up the final yield.

In the case of swedes, however, where this effect does not enter, rotary cultivation was often associated with a reduced yield, of consider-

able magnitude in the 1926 experiments, and smaller but still significant in the 1928 experiments. This crop is known to be sensitive to soil conditions, and possibly the 1928 results may be due to some small factor that can be removed after further experience of rotary cultivation. The cause of the large reduction in yield in 1926 has already been discussed. It was due to an excessive and extensive hardening or "capping" of the soil on the rotary cultivation plots, which, no doubt, would have seriously affected most agricultural crops.

The results of the Rothamsted experiments are confirmed by the work of Gade(4) in Germany. His experiments were made on various soils and crops, including winter wheat, barley, oats, sugar beet and potatoes. The yields for sugar beet were the same on the rotary cultivated and ploughed plots; with the other crops a slight and insignificant increase was given; in one of the potato experiments a significant increase was obtained on the rotary cultivated plots. Gade also confirmed the initial superiority of rotary cultivated soil, and was able to show by measurements of pore volume that it existed in a much looser condition than soils cultivated by normal methods. Owing to the presence of numerous stones and flints in the Rothamsted soil, no attempt was made to take volume measurements. The effect of rotary cultivation was, however, visible to the naked eye. The soil rapidly settles into a compacter tilth, which in some cases (*e.g.* the 1926 experiments) may be deleterious to the growing crop. In Gade's experiments the initial lightness of structure had disappeared after 100 days. The absence of any marked benefits in yield after rotary cultivation is, therefore, not surprising. On the other hand, there is no foundation for the belief that a very fine tilth is normally produced by the rotary cultivator. Such a tilth would almost always be disadvantageous on heavy loams and clay soils. The action of the tines ensures that few large lumps of soil are left and the tilth is, therefore, more uniform, but there is no great preponderance of the smallest sized lumps.

The experiments clearly show that the tilth of medium and heavy soils depends predominantly on the weather conditions, the type of implement employed having normally only a secondary influence. The rotary cultivator is of greatest service when the soil is not in good condition, as is the case after a wet and open winter. In these circumstances, it is difficult to refine the soil to a good tilth, and here rotary cultivation shows to the best advantage, particularly if it is combined with the operation of ridging, which is in itself an effective method in the same conditions.

SUMMARY.

Experiments extending over the four years, 1926–9 inclusive, have been carried out on a heavy and stony loam soil to compare rotary cultivation with normal methods for the production of a seed-bed. The work has been confined to spring-sown crops—swedes and barley—and to spring cultivations.

Rotary cultivation gives earlier and better germination of seed, followed by better early growth.

In every experiment, however, the final yield has either been no better, or else significantly below that obtained from the plots cultivated in the usual way. The barley crop gives equally good yields under the various soil cultivation treatments; although there is better germination under rotary cultivation, the plants on the remaining plots, having greater root range, throw out more tillers and thus level up the yield. The swede crop did not do so well under rotary cultivation, in spite of better early growth. In the 1926 experiments this was due to an extensive hardening or "capping" of the soil on the rotary cultivation plots but, although this effect was absent in the 1928 experiments, a reduced yield was still obtained.

In addition to records of yield, measurements were made of the amount of soil pulverisation or comminution produced by the various implements. Samples of the soil before and after cultivation were passed through a set of sieves, of different mesh, and the proportion of soil on each sieve was recorded.

The meteorological factors exercise a predominating influence on the physical condition of the soil; the influence of the implement is secondary.

Rotary cultivation is most effective on unkindly soil, *e.g.* after a wet and open winter, as it leaves a smaller proportion of large lumps.

It does not produce an appreciably finer tilth than the usual implements, but it leaves the soil in a much looser condition, and thus encourages the early germination of seeds.

The looser tilth becomes more compact with time, and the initial advantage of rotary cultivation then disappears.

If a ridging body is attached behind the rotary cultivator, a further comminution of the soil is produced. The operation of ridging is itself surprisingly effective in breaking up land in poor physical condition.

Ploughing does not necessarily produce any comminuting action on the soil unless it is well supplied with humus and is, therefore, in good

inherent tilth. When the soil is in poor condition, there is a tendency for the smallest soil crumbs to be aggregated into larger ones, probably owing to the compression of the soil by the tail-end of the mould board. This effect counteracts the increase of surface due to the sub-division of the largest sized lumps.

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(*Received March 12th*, 1930.)

DECOMPOSITION OF KERATIN BY SOIL MICRO-ORGANISMS.

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(With One Text-figure.)

Among the so-called scleroproteins the keratins occupy a prominent place as physiologically important compounds of wide distribution in the animal kingdom, where they occur in horns, hoofs, nails, feathers, hair, epithelial cells, etc. They are chemically characterised by their richness in the amino acid cystine, which gives them a high sulphur content (up to 15 per cent.), further by being very little soluble in chemical reagents, and by their great resistance to the action of proteolytic enzymes. Although an enormous amount of work has been devoted to the study of the decomposition of proteins by various micro-organisms, this never seems to have been extended to the decomposition of keratins. That keratin-decomposing micro-organisms exist can be concluded from the fact that horn and hoof meal, when added to the soil as fertilisers, undergo a fairly rapid decomposition which results in the formation of ammonia and nitrate. Pfeiffer(5) thus found in pot experiments that horn meal with 12·3 per cent. N had a fertilising value corresponding to 83 per cent. of that of sodium nitrate, and that 59–62 per cent. of the N of the horn meal was utilised by the plants in 3 years. Popp(6) found in field experiments a corresponding fertilising value of 70 per cent., and in laboratory experiments, where horn meal with 13·1 per cent. N was allowed to decompose in moist soil, he found that after 12 weeks 58–59 per cent. of the N of the horn had been transformed into ammonia and nitrate. As to the action of pure cultures of micro-organisms on horn, we have but very scant information. H. M. Ward(9) observed the fungus *Onygera equina* growing on horn with a marked formation of ammonia; under certain conditions the fungus would also grow on horn shavings in pure culture. Benecke(1) grew the chitin-decomposing *Bac. chitinovorus* in mineral solution with horn meal, and found a vigorous growth continuing when the solution was repeatedly renewed, from which he concluded that the keratin was probably attacked. Münter(4) found that seven soil actinomycetes in pure culture produced large amounts of

ammonia from casein, peptone and glue, but only very little from horn meal.

The present paper records a small experiment on decomposition of keratin, carried out in connection with some more extensive studies on decomposition of farmyard manure in soil. Keratin was prepared from horn meal containing 14·5 per cent. N in the following way: 10 gm. of horn meal were treated with 100 c.c. of ether to remove the fats; after filtering and drying the material was digested for 4 days at 37° C. with 200 c.c. of a 0·5 per cent. pepsin solution, the reaction of the mixture being adjusted to *p*H about 1·5 by means of hydrochloric acid. After filtering and careful washing with distilled water the material was further digested with 200 c.c. of a 0·5 per cent. trypsin solution (*p*H about 9 with sodium hydroxide) for 3 days at 37° C. The residue was then washed, first with water, then with dilute hydrochloric acid, finally with distilled water till free from acid, and dried at 55° C. About 4 gm. of keratin were obtained in this way. The air-dry material contained 13·43 per cent. N and 46·5 per cent. C.

With this substance decomposition experiments were carried out in two soils. The first was a heavy clay soil, poor in organic matter, of *p*H 6·4, from an unfertilised plot on Hoos Field, the second a clayey garden soil, rich in organic matter, of *p*H 7·2. Soil portions of 200 gm. received additions of 24–25 per cent. of distilled water, and some received 0·5 per cent. keratin (on air-dry basis). The soils were incubated at 25° C. in reagent bottles of about 500 c.c. capacity, closed with tight-fitting corks through which passed a glass tube filled with cotton wool. Incubation was carried on for 120 days, during which time the moisture content was kept as constant as possible, and counts of bacteria and actinomycetes as well as estimations of ammonia and nitrate were carried out repeatedly. Counts of bacteria and actinomycetes were made by plating at a dilution of 1 : 250,000–1 : 500,000 on the following medium: dextrose 2·0 gm., casein dissolved in 10 c.c. 0·1 *N* NaOH 0·2 gm., K_2HPO_4 0·5 gm., $MgSO_4$ 0·2 gm., agar 15 gm., distilled water 1000 c.c., *p*H 6·5–6·6. Five parallel plates were incubated at 20° C. for 10 days. Nitrate was determined by the Devarda method, ammonia by Bengtsson's method: repeated extraction with 0·5 *N* KCl solution and distillation with MgO. The results are found in Table I. The addition of keratin has had a comparatively slight influence on the numbers of bacteria; only in the Hoos Field soil after 40 days is there a significant increase, but whether this is a primary effect or due to bacteria utilising the decomposition products of the keratin cannot be ascertained. The

fluctuations in the numbers in the garden soil are only what is always observed when soil is air-dried and re-moistened. The actinomycetes, on the other hand, are stimulated to a marked extent, especially in the garden soil, where their "numbers" even surpass those of bacteria. Plate counts of actinomycetes do not necessarily indicate the numbers of individual organisms, since they consist of filaments which, on spore formation, break up into oidia; but since spore formation depends on

Table I. *Development of bacteria and actinomycetes and production of ammonia and nitrate in soils with addition of keratin.*

	Hoos Field soil							
	Control soil				Soil + 0·5 % keratin			
Time	Bact.*	Act.*	NO_3-N†	NH_4-N†	Bact.*	Act.*	NO_3-N†	NH_4-N†
Start	28·7	2·1	1·4	0·4	28·7	2·1	1·4	0·4
20 days	62·3	2·3	5·0	0·0	85·2	7·2	4·5	5·4
40 ,,	55·0	2·6	9·1	0·0	160·4	21·2	16·8	Trace
120 ,,	35·1	2·1	6·6	0·0	37·6	12·0	30·1	0·0
	Percentage of added keratin-N nitrified: 35.							
	Garden soil							
Start	13·8	1·2	2·8	0·0	13·8	1·2	2·8	0·0
10 days	—	—	—	—	19·6	20·8	—	—
20 ,,	—	—	—	—	32·4	43·8	25·2	0·0
40 ,,	—	—	9·5	0·0	—	—	—	—
60 ,,	—	—	—	—	14·4	34·6	32·2	0·0
120 ,,	15·4	2·6	12·8	0·0	9·3	28·3	39·2	0·0
	Percentage of added keratin-N nitrified: 39.							

* Millions per gm. of moist soil.
† Milligrams per 100 gm. of air-dry soil.

previous vegetative growth, the increase in colony numbers still indicates that active growth has taken or is taking place. After 20 days there is a good production of mineral nitrogen; in the Hoos Field soil there is a temporary accumulation of ammonia, whereas in the garden soil the ammonia appears to be nitrified as soon as formed. Fig. 1, where the total amounts of mineral N are plotted against the time, shows that the nitrification of keratin-N, unlike that of soluble protein, is slow but steady, so that after 4 months, when 35–39 per cent. of the added nitrogen has been nitrified, there is still no slowing down of the process. It is interesting to compare this with the observation of Waksman (7, 8), that production of ammonia from proteins by actinomycetes is mostly slow, but continuous, so that on prolonged incubation large amounts of ammonia will accumulate. In the present instance, the association of an increase in the actinomycete flora with the nitrification of the keratin suggests that these organisms are of primary importance in the process. The keratins, which are so little soluble and so unusually

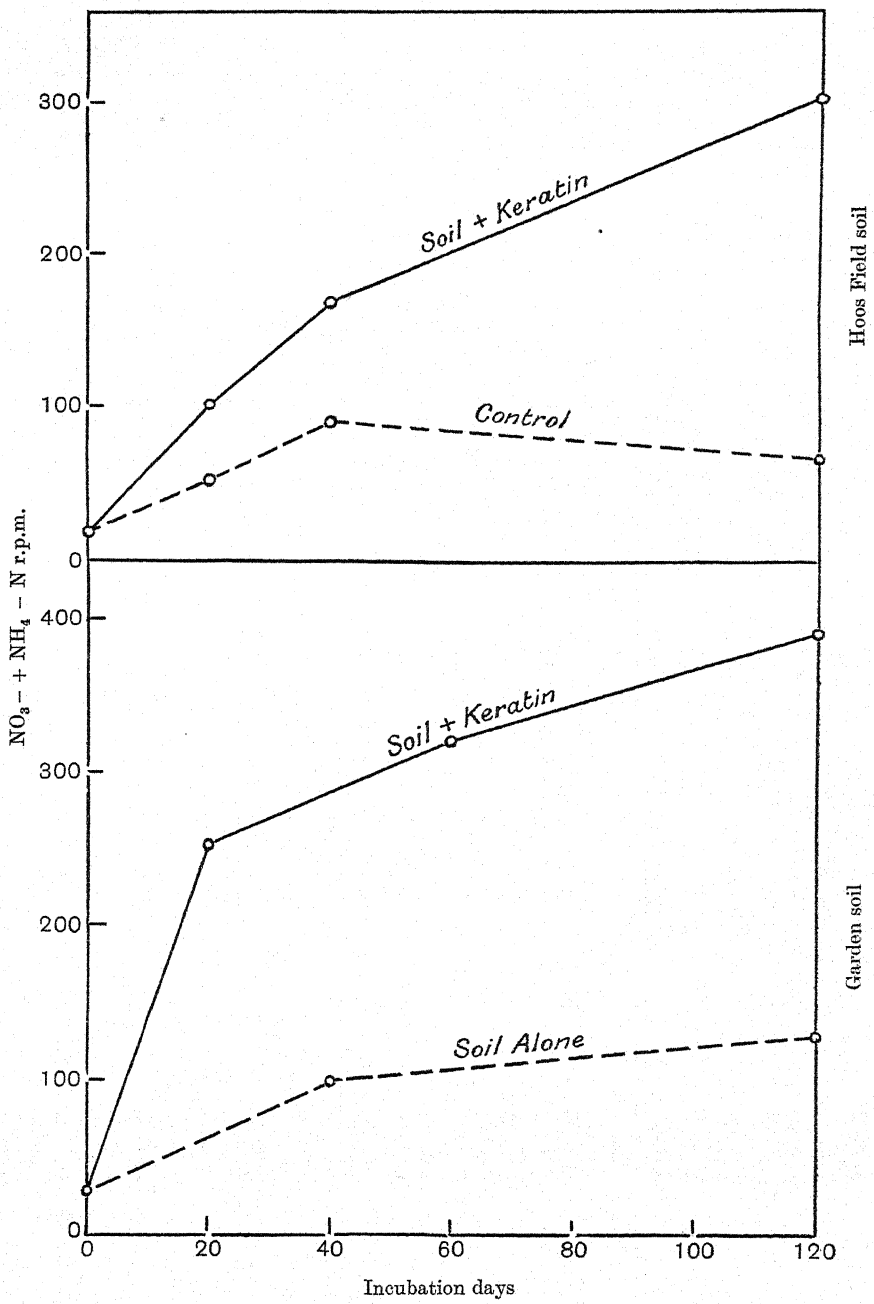


Fig. 1. Decomposition of keratin in Hoos Field and garden soil.

resistant to the action of the proteolytic enzymes of the digestive tract, thus appear to be readily attacked by the soil micro-organisms, although their decomposition is much slower than that of soluble proteins.

The increases in numbers of actinomycete colonies were, especially in the garden soil, almost entirely due to organisms of two distinct types, one with pale rose and one with lead-grey aerial mycelium. Two strains representing these two types were isolated and grown in pure culture on sterile sand with addition of keratin. Small round flasks of 50 c.c capacity were used, containing 50 gm. of pure quartz sand, 0·25 gm. of keratin, and 15 c.c. of a solution of 0·1 per cent. K_2HPO_4 + 0·05 per cent. $MgSO_4$. After sterilisation and inoculation, the flasks were incubated for 30 days at 25° C. Both strains made a good growth and produced plainly visible rose and grey aerial mycelia. Within about a week the horny smell of the keratin disappeared and was replaced by the characteristic earthy odour produced by most soil actinomycetes. At the end of the experiment ammonia was determined with the following result:

Culture	NH_4 – N mgm. per 50 gm. of sand
I (rose aerial mycelium) ...	5·9
II (grey ,,) ...	5·2

The addition of 0·25 gm. keratin corresponds to 33·6 mgm. of nitrogen, of which thus 16–17 per cent. (+ the amount which may have been lost through evaporation of ammonia) had been ammonified by the actinomycetes.

Attempts were made to identify the organisms with previously described species. Their morphology was studied on casein-dextrose-agar and, for observation of cultural characters, they were grown on the following media:

1. *Czapek's agar* (Waksman (7)): saccharose 30·0 gm., $NaNO_3$ 2·0 gm., K_2HPO_4 1·0 gm., $MgSO_4$ 0·5 gm., KCl 0·5 gm., $FeSO_4$ trace, agar 15 gm., H_2O 1000 c.c., *p*H 7·0.

2. *Dextrose agar* (Krainsky (3)): dextrose 10·0 gm., asparagin 0·5 gm., K_2HPO_4 0·5 gm., agar 15 gm., H_2O 1000 c.c., *p*H 7·0.

3. *Glycerin agar* (Conn (2)): glycerin 10·0 gm., Na-asparaginate 0·5 gm., K_2HPO_4 0·5 gm., agar 15 gm., H_2O 1000 c.c., *p*H 7·0.

4. *Starch-casein-agar*: soluble starch 10·0 gm., casein dissolved in 1 *N* NaOH 1·0 gm., K_2HPO_4 1·0 gm., $MgSO_4$ 0·5 gm., agar 15 gm., H_2O 1000 c.c., *p*H 7·0.

5. *Nutrient agar*: meat extract 5·0 gm., peptone 10·0 gm., NaCl 5·0 gm., agar 15 gm., H_2O 1000 c.c., *p*H 7·0.

6. *Dextrose broth*: 5 without agar, plus 1 per cent. dextrose.
7. *Potatoes.*
8. *Milk.*
9. *Synthetic solution*: dextrose 10·0 gm., $NaNO_3$ 1·0 gm., K_2HPO_4 0·5 gm., $MgSO_4$ 0·5 gm., H_2O 1000 c.c.

The agar media were used for slope cultures. Aerial mycelium from young dextrose agar (No. 2) cultures served as inoculum, and all cultures were incubated at 30° C., at which temperature both organisms made an excellent growth.

ACTINOMYCES I.

Morphological characters.

Vegetative mycelium of the usual actinomycete type. Aerial mycelium develops after a few days, consisting of long, fairly straight, slightly branched hyphae, 1·0μ broad, often showing a wavy effect, but not producing regular spirals. Hyphal branches break up into oidia, 1·0–1·3 × 1·2–1·6μ.

Cultural characters.

Czapek's agar. Good growth. Vegetative mycelium superficially spreading, at first (3 days) smooth and very light ochre-yellow, later (7–12 days) becoming much wrinkled and lemon-yellow. Aerial mycelium begins to develop after a few days, first along edges, later (12–15 days) covering all growth, at first white, later with a slight pink tinge. A lemon-yellow soluble pigment begins to develop after 7–10 days.

Dextrose agar. Fair growth. Vegetative mycelium flat, superficial, first cream-coloured, later turning light ochre-yellow. Aerial mycelium develops very early as a smooth, even layer, first white, after 5–7 days faint pink. No soluble pigment.

Glycerin agar. Fair growth. Vegetative mycelium smooth, narrow, spreading deep into medium, first cream coloured, later changing from light ochre-yellow to faint orange (12 days). White aerial mycelium develops early, covering the growth. No soluble pigment.

Starch-casein-agar. Slope culture: good growth. Vegetative mycelium raised, narrow, growing deep into medium, lemon-yellow with greenish tinge. Aerial mycelium develops early, first thin, later abundant, after 12 days of a very characteristic pink colour. Light lemon-yellow soluble pigment.

Plate culture. 6 days: good growth. Colonies compact, a little raised, with ochre-yellow reverse and cottony, pink aerial mycelium.

Treatment with iodine solution shows diastatic zones, 2–3 mm. wide, around colonies.

Nutrient agar. Good growth. Vegetative mycelium flat, superficial, cream coloured. No aerial mycelium. No pigment.

Dextrose broth. 7 days. Cream-coloured surface ring and colonies. No pigment.

Potato. Vegetative mycelium spreading, wrinkled, first greyish-yellow, later (7 days) yellowish brown. No aerial mycelium. No soluble pigment.

Milk. Cream-coloured surface ring, after 7 days sulphur-yellow. No aerial mycelium. No soluble pigment. Milk is not coagulated, but slowly cleared (completely after 15 days).

Synthetic solution. 7 days. Colourless colonies on bottom of tube. No pigment. No nitrite formation.

This organism corresponds fairly well to the description of *Actinomyces citreus* Krainsky, as given by Waksman(7), who mentions that sometimes a white aerial mycelium is produced on Czapek's agar instead of the typical yellow. Waksman observed spiral formation in aerial mycelium on dextrose agar and found no soluble pigment in Czapek's agar, but these characters, as well as others, may be subject to some variation.

Actinomyces II.

Morphological characters.

Vegetative mycelium of the usual actinomycete type. Aerial mycelium consists of rather short, profusely branched, much bent and curved hyphae, 1·3 μ broad, often with a tendency to curl, but not forming real spirals. Hyphae break up into oidia, 1·1–1·3 × 1·2–1·6 μ.

Cultural characters.

Czapek's agar. Very scant growth. Vegetative mycelium thin, spreading deep into medium, colourless. Thin veil of aerial mycelium, first white, later (7–12 days) light grey. No pigment.

Dextrose agar. Good growth. Vegetative mycelium smooth, narrow, a little raised, first colourless, later (9–14 days) with yellowish grey reverse. Aerial mycelium develops early, abundant, after 3 days greyish with white edges, later lead-grey. No pigment.

Glycerin agar. Good growth. Vegetative mycelium smooth, narrow, a little raised, cream-coloured. Aerial mycelium thick, white, cotton-like. No pigment.

Starch-casein-agar. Slope culture: excellent growth. Vegetative mycelium smooth, spreading, cream-coloured, after 7–12 days with dark grey reverse. Aerial mycelium abundant, ash-grey. No pigment.

Plate culture. 6 days: good growth. Colonies flat, spreading, with cream-coloured reverse. Aerial mycelium abundant, ash-grey. Treatment with iodine solution shows diastatic zones of 5–6 mm. around colonies.

Nutrient agar. 7 days. Excellent growth. Vegetative mycelium smooth, superficial, a little raised, yellowish grey, with thin greyish white aerial mycelium on centre of growth. Deep brown soluble pigment.

Dextrose broth. 7 days. Cream-coloured surface ring with trace of white aerial mycelium, colourless floating colonies. Light brown pigment. Reaction to brom-cresol-purple changed to faintly acid.

Potato. Good growth. Vegetative mycelium raised, much wrinkled, dark yellowish grey. No aerial mycelium. Potato plug coloured greyish black.

Milk. Brown surface ring, after 7 days covered with thin white aerial mycelium. Milk is not coagulated, but slowly cleared up from above (completely after 15 days). Dark brown pigment.

Synthetic solution. 7 days. Good growth. Colourless colonies attached to sides of tube or swimming on surface, the latter ones covered with grey aerial mycelium. No pigment. No nitrite formation.

This strain resembles very much an unnamed form described by Waksman(7) as *Actinomyces* 145, from which it differs only in its production of a brown soluble pigment on nutrient agar and its action on milk, which indicates a good proteolytic activity—characters which are both subject to variation. However, since only one single strain was available, it was not deemed advisable to name it as a new "species."

SUMMARY.

Keratin, prepared from horn meal, was added to moist field and garden soil and allowed to decompose in the laboratory. The keratin was found to undergo a decomposition resulting in a slow, but steady accumulation of ammonia and nitrate. 35–40 per cent. of its nitrogen was transformed into nitrate after 120 days. The addition of keratin produced little or no increase in the number of bacterial colonies on agar platings, but markedly increased the number of actinomycete colonies, especially in garden soil. Two strains of actinomycetes were isolated and found capable of thriving on keratin in pure culture, decomposing the keratin

with the formation of ammonia. One of the strains could be recognised as *Actinomyces citreus* Krainsky, as described by Waksman. The other strain could not be named, but corresponded closely to the description of Waksman's *Actinomyces* 145.

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(*Received March 29th*, 1930.)

A METHOD OF ESTIMATING THE YIELD OF A MISSING PLOT IN FIELD EXPERIMENTAL WORK.

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It is essential in field experiments that the greatest care should be taken, so that reliance can be placed on the yield figures obtained, and normally there should be no case for rejection of any single plot. Yet it sometimes happens that the recorded yields are imperfect, owing to causes beyond the control of the experimenter. Thus a plot yield may be completely missing, or the plant may be so poor on a plot, owing to disease or for other reasons, that the recorded yield is not comparable with that of the other plots. Each such case should be carefully considered on its merits before a decision is reached as to whether the yield should be rejected or not. More than one lost plot would seriously jeopardise the accuracy of the experiment, and where modern methods of arrangement (Randomised Blocks and the Latin Square) are used, even one missing plot will disturb the symmetry and balance of the arrangement. In the former design, one plot of each treatment occurs in each block of land, and the variation between blocks as a whole is a measure of the differences in soil fertility over the experimental area. In the case of the Latin Square there are as many replicates as there are treatments, so arranged that no treatment occurs more than once in each row and column of the square.

Now when only one plot is missing something can be done in the way of estimating a value for it from the other plots, although it should be emphasised that there will be always some loss in accuracy as compared to an experiment with the full complement of plots. Cases that have occurred are: (*a*) a weight was missed from one plot of a Latin Square; (*b*) the corner plot of a similar square gave a very poor yield, the reason being that it adjoined the farm road and had evidently suffered badly from trampling. There was thus a good case for rejection of the particular plot; (*c*) in figures for a randomised block experiment recently submitted to this department, one figure, a protein determination, was missing and could not be retrieved.

Naturally we want to get as much information as possible from the experiment, even though it has suffered in the direction indicated. We could delete the whole block containing the missing plot, or the row or

column of the Latin Square, and this was actually done at a recent hay trial when it was found that one of the columns had suffered from the depredation of cows which had broken through the fence. Clearly this method sacrifices a good deal of information that the plots rejected would have been capable of furnishing, and what is needed is a means of utilising all the known plot values to form a best estimate of the missing yield. When only one plot is missing, such a method can be found by the application of the linear law which is the foundation of the analysis of variance procedure. For example in a randomised block experiment, if the yield of a particular plot is x, then the deviation of x from its mean $\bar{x}$ over the whole experiment is regarded as made up of a part due to the block in which it is situated, together with a part due to the treatment on that particular plot.

Thus $Y = b_p + t_q$, where Y is the supposed true deviation from the mean yield of the plot having treatment q in block p. For example, consider the following table of potato yields in lb.

	Treatments					
Block	1	2	3	4	Mean	
A.	139·0	219·0	200·5	145·0	175·88	
B.	197·5	205·0	206·0	182·5	197·75	
C.	156·0	229·5	210·0	245·5	210·25	
Mean	164·17	217·83	205·50	191·00	194·63	General mean

The linear law postulates that the yield of treatment 1 on Block A. is made up of mean yield of Block A. + mean of treatment 1 − general mean, *i.e.* 145·4. Actually it is 139·0, and the difference 6·4 represents the error. The sum of the squares of all such differences is, in fact, the sum of squares due to error in the analysis of variance. For the Latin Square there will be a part due to the row in which a particular plot is situated, a part for column, and a part for treatment, so that in this case the law is $Y = r_p + c_q + t_s$. Assuming such a linear law, we can now find an estimate for the missing yield.

(*a*) Randomised Blocks.

The yield Y depends on the block factor b and the treatment factor t. Let $Y = b_p + t_q + k$ be the function, where k is constant throughout, b changes from block to block, and t from treatment to treatment, and let $b = 0$ in the block which contains the missing plot, and $t = 0$ for the treatment of which the missing one is a replicate, so that k is actually the estimated yield of the missing plot, and b and t represent deviations from it due to change in block and treatment in the other plots.

Suppose there are s treatments and n replicates of each. Then we have $ns - 1$ actual yields y, and we want to determine the constants so that the sum $S(y - Y)^2$ is a minimum taken over all the $ns - 1$ plots whose values are known. That is, we are to make $S(y - b_p - t_q - k)^2$ a minimum for variations of the constants b_p, t_q and k.

Minimising for variation in k gives

$$\overset{ns-1}{\underset{1}{S}}(y - b_p - t_q - k) = 0,$$

i.e.

$$\overset{ns-1}{\underset{1}{S}}(y) = sB + nT + (ns - 1)k = S, \text{ say,} \qquad \ldots\ldots(1)$$

where the summation extends over all the $ns - 1$ plots, and

$$B = b_1 + b_2 + \ldots + b_n, \quad T = t_1 + t_2 + \ldots + t_s,$$

it being remembered that B contains only $(n - 1)$ terms and T only $(s - 1)$ terms, one being missed from each since $b = 0$ for one block and $t = 0$ for one treatment, as was postulated above.

Minimising for variation in b_p, we get

$$\overset{s}{\underset{1}{S}}(y) = sb_1 + T + sk = y_1, \text{ say,}$$

where the summation extends over the s plots in the first block,

$$\overset{s}{\underset{1}{S}}(y) = sb_2 + T + sk = y_2 \text{ for summation over block 2,}$$

and so on for $(n - 1)$ blocks, omitting that in which $b = 0$. Adding these $(n - 1)$ equations, we get for the sum over $(n - 1)$ blocks

$$\overset{n-1}{\underset{1}{S}}(y_p) = sB + (n - 1)T + s(n - 1)k. \qquad \ldots\ldots(2)$$

Similarly, minimising for variations in t_q, we have, for the sum over $(s - 1)$ treatments,

$$\overset{s-1}{\underset{1}{S}}(y_q) = (s - 1)B + nT + (s - 1)nk, \qquad \ldots\ldots(3)$$

where y_q represents the sum of all the replicates of treatment t_q. The equations (1), (2) and (3) serve to determine B, T and k, and the contributory equations will give the values of b_p and t_q. It is sufficient for our purpose to find k. From the above equations we get

$$k(n - 1)(s - 1) = (n + s - 1)S - s.S_t - n.S_b, \quad \ldots\ldots(A)$$

where

S_t = sum of treatment totals not including the treatment of which one plot is missing,

S_b = sum of block totals not including the block with the missing plot,

S = sum of all the known $(ns - 1)$ plot yields.

With the estimated value k for the missing plot we can proceed as usual with the analysis of variance. One degree of freedom has been lost from those for error due to fitting the value k, and the degrees of freedom now are: Blocks $n-1$; Treatment $s-1$; Error $(n-1)(s-1)-1$; Total $ns-2$.

Example. We can illustrate the process by the case referred to at the beginning, in which a figure was missing from a set of calculations of protein percentage in peas. There were nine different treatments and eight replicates.

	Treatments									
Block	1	2	3	4	5	6	7	8	9	Totals
A.	15·96	16·80	19·08	21·15	21·14	23·02	20·60	26·34	25·32	189·41
B.	21·62	24·44	26·33	[20·93]	20·22	20·87	19·21	20·04	21·30	[194·96]
C.	18·78	18·36	19·40	20·40	20·81	23·77	17·24	18·00	20·46	177·22
D.	20·18	20·52	20·42	19·96	20·69	20·90	21·68	21·90	20·68	186·93
E.	16·20	16·90	19·81	17·21	19·59	21·22	18·72	20·04	19·02	168·71
F.	18·64	20·33	22·71	19·29	18·33	19·06	20·04	21·31	22·19	181·90
G.	18·89	23·60	22·42	16·91	15·40	17·00	16·71	18·02	19·84	168·79
H.	19·22	20·47	20·98	18·06	20·34	21·38	16·83	15·66	17·21	170·15
Total	149·49	161·42	171·15	[153·91]	156·52	167·22	151·03	161·31	166·02	[1438·07]
Mean	18·686	20·178	21·394	19·239	19·565	20·903	18·879	20·164	20·753	19·973

The treatments in order from left to right are:

(1, 2, 3) No potash without superphosphate, and with single and double dressings of superphosphate.

(4, 5, 6) Single dressing of potash without superphosphate, and with single and double dressings of superphosphate.

(7, 8, 9) Double dressing of potash without superphosphate, and with single and double dressings of superphosphate.

The figures enclosed in brackets consist of the calculated value for the missing figure corresponding to treatment 4 on Block B., and the totals with this value included.

The equations for determining k are:

$$S = 1417{\cdot}14 = 71k + 9B + 8T, \qquad \text{from (1)}$$
$$S_b = 1243{\cdot}11 = 63k + 9B + 7T, \qquad \text{from (2)}$$
$$S_t = 1284{\cdot}16 = 64k + 8B + 8T, \qquad \text{from (3)}$$

and solving as in the general case, these give

$$k = \tfrac{1}{56}\{16 \times 1417{\cdot}14 - 9 \times 1284{\cdot}16 - 8 \times 1243{\cdot}11\}$$
$$= 20{\cdot}93.$$

Now put in this value of k for the missing figure in the table, and proceed

with the analysis of variance. There would in general be 71 degrees of freedom for a randomised block experiment with nine treatments and eight blocks, and the number here is therefore 70. The analysis of variance is now:

	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	7	80·33	11·48	—
Treatments:				
Potash	2	0·48	0·24	—
Superphosphate ...	2	52·17	26·08	—
Interaction ...	4	3·93	0·98	—
Error	55	252·36	4·59	*0·437 or 2·19 %
Total	70	389·27	—	—

* Mean of 24 plots.

The experiment thus shows a significant effect of superphosphate in increasing the protein percentage of the peas, but no effect of potash, and the three superphosphate means (of 24 plots each) are 18·93, 19·97 and 21·02 respectively, with a standard error of 0·437.

(*b*) LATIN SQUARE.

The procedure for the Latin Square is similar, but we now write

$$Y = r_p + c_q + t_s + k$$

to show dependence on row, column and treatment. As before, k is the value of Y for the missing plot. Suppose there are n treatments, so that $n^2 - 1$ plot yields are known when one is missing.

$$S\,(y - r_p - c_q - t_s - k)^2$$

is now minimised, yielding the following equations.

For variations in k, summing over the whole area except the missing plot,

$$\overset{n^2-1}{\underset{1}{S}}\,(y) = (n^2 - 1)\,k + nC + nR + nT = S, \text{ say,} \quad \ldots\ldots(1)$$

where for convenience C has been written for the sum of all quantities like c_q, and so on for R and T. For columns, we have $(n - 1)$ equations of the type

$$\overset{n}{\underset{1}{S}}\,(y) = nk + nc_q + R + T = y_q, \text{ say,}$$

where the sum extends over column q. On addition these give

$$\overset{n-1}{\underset{1}{S}}\,(y_q) = (n - 1)\,nk + nC + (n - 1)\,R + (n - 1)\,T, \quad \ldots\ldots(2)$$

and similarly for the sum over $(n-1)$ rows

$$\sum_{1}^{n-1}(y_p) = (n-1)\,nk + (n-1)\,C + nR + (n-1)\,T, \quad \ldots\ldots(3)$$

and for treatments

$$\sum_{1}^{n-1}(y_s) = (n-1)\,nk + (n-1)\,C + (n-1)\,R + nT. \quad \ldots\ldots(4)$$

The equations (1), (2), (3) and (4) may now be solved for k, C, R and T. The solution in terms of row, column and treatment totals reduces to

$$k = \frac{1}{n-1}S_1 + \frac{2}{(n-1)(n-2)}S_2, \qquad \ldots\ldots(B)$$

where S_1 represents the sum of the yields of the $3(n-1)$ plots which agree with the missing plot in row, column or treatment, and S_2 is the sum of the plots which do not so agree.

An interesting illustration is given by a sugar beet experiment which was laid out in a 5×5 Latin Square. The yields are given in lb. for each plot of $\frac{1}{40}$ acre[1].

A. 306	B. 556	C. 369	D. 332	E. 396
B. 357	E. 485	D. 358	C. 317	A. 325
C. 309	D. 467	E. 367	A. 275	B. 413
D. 418	A. 453	B. 389	E. 324	C. 335
E. 503	C. 572	A. 346	B. 397	D. [279]

The yield in the bottom right-hand corner is markedly low, especially when we consider the relatively high yields for treatment D on the other plots; and actually a note was appended with the figures commenting on the very poor plant on this particular plot and saying that it was due to trampling during the growing season. Accepting the figure of 279 the analysis of variance was as follows:

	Degrees of freedom	Sums of squares	Mean square	$\frac{1}{2}\log_e \times$ Mean square	Standard error
Rows	4	9,279·04	2,319·76	—	—
Columns	4	97,905·44	24,476·36	—	—
Treatment	4	22,266·64	5,566·66	4·3123	—
Error	12	28,384·72	2,365·39	3·8843	*21·75 or 5·64 %
Total	24	157,835·84	6,576·49	—	—

* Mean of 5 plots.

[1] See Wishart and Hines, "Fertiliser Trials on the Ordinary Farm," *J. Min. Agric.*, Sept. 1929, pp. 524–32.

If this was all that could be done, we should be forced to conclude that the differences between the treatment means are not significant in the light of their standard error. Let us, however, discard the misleading value 279 and replace it by a value estimated on the assumption of the linear law above. We find for the estimated value,

$$k = \tfrac{1}{4}.(4862) - \tfrac{1}{6}.(4507)$$
$$= 464{\cdot}3.$$

Take this as 464, since all the weights are only accurate to the nearest pound. One degree of freedom is lost, and the analysis of variance becomes

	Degrees of freedom	Sums of squares	Mean square	$\frac{1}{2}\log_e \times$ Mean square	Standard error
Rows	4	27,142·64	—	—	—
Columns	4	89,943·04	—	—	—
Treatment	4	22,148·24	5,537·06	4·3096	—
Error	11	11,897·52	1,081·59	3·4931	*14·71 or 3·74 %
Total	23	151,131·44	—	—	—

* Mean of 5 plots.

The part due to error is now so much smaller that, despite the fact that the number of degrees of freedom is reduced, the z test shows that there is a significant response to treatment[1]. The value of z is 0·8165, and since a value of z greater than 0·6055 occurs by chance in only 5 per cent. of cases, this value is certainly significant. Thus it has been possible to gain positive results from an experiment which at first appeared to have been spoilt by the unreliable figure for the yield of one plot. The results are:

Average yield	No nitrogen	Sulphate of ammonia	Muriate of ammonia	Cyanamide alone	Cyanamide and nitrate of soda	Mean	Standard error
Tons per acre	6·1	7·4	7·3	6·8	7·5	7·0	0·26
Per cent.	86·7	105·5	103·7	96·7	107·4	100·0	3·74

The other possible procedure, of discarding the whole of the last column, in which the damaged plot occurred, and treating the remainder as a randomised block experiment, gives, like the first analysis, a standard error so high that nothing could be inferred from the experiment. We could not, indeed, expect much from such an experiment with only four replicates. This last procedure is moreover open to the objection that the four columns used are not in fact randomised blocks, but are subject to the further restriction necessary to make them part of a Latin Square.

[1] See Wishart and Hines, *loc. cit.*

SUMMARY.

In cases of field experiments when one plot is missing, a method has been developed for furnishing an estimate of the yield of the missing plot, based on all the other values. The calculation is given for (*a*) a Randomised Block experiment, and (*b*) a Latin Square arrangement. In both cases the actual arithmetic is very simple.

The steps in the procedure are:

(1) Determine the desired value by an application of the equation (*A*) or (*B*) according as the experiment was arranged in Randomised Blocks or in a Latin Square.

(2) Proceed as usual with the analysis of variance, using the estimated figure for the missing yield, and remembering to deduct one from the number of degrees of freedom ascribable to error.

(*Received March 29th*, 1930.)

"SINGLE VALUE" SOIL PROPERTIES. A STUDY OF THE SIGNIFICANCE OF CERTAIN SOIL CONSTANTS.

III. NOTE ON THE TECHNIQUE OF THE KEEN-RACZKOWSKI BOX EXPERIMENT.

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(With Three Text-figures.)

In Paper II of this series(2), one of the simple physical measurements proposed for the preliminary examination of a soil was the perforated box experiment described by Keen and Raczkowski(5). In Keen and Raczkowski's original experiments, rectangular boxes were used, while in the measurements on Natal soils quoted in Paper II the boxes were cylindrical, 1 in. in radius[1] and 1 in. deep. The box was formed by a 1 in. length of brass tubing, of 2 in. diameter (which will be referred to as the collar), fitting tightly into the base, which consisted of a second piece of tubing, $\frac{5}{8}$ in. long, carrying the perforated bottom. The collar, when placed in position, also held the filter paper in position in the base.

This form of box was not very convenient; it happened not infrequently that a few soil particles lodged between the collar and the walls of the base, with the result that, at the end of an experiment, violent methods were needed to separate the collar from the base. Hence, after the box had been in use for a few weeks, the bottom became so misshapen that the internal volume could no longer be determined by simple measurements of the depth and diameter.

To overcome this difficulty, the procedure was modified by abandoning the use of the collar altogether, and using the base only. The filter paper was kept in position by inserting a split brass ring, of about the same diameter as the base, in the bottom of the box. The effective internal volume of the box was obtained by subtracting from the volume calculated from its cylindrical form the volume (*i.e.* mass/density) of the ring. This correction amounted to about 0·2 c.c., while the total volume of the box was about 30 c.c. This method worked very well, and it is proposed to use the modified form of box just described in any future work.

The main value of the Keen-Raczkowski experiment is for com-

[1] Not 1 in. diameter, as stated erroneously in Paper II.

parison of the properties of different soils under similar experimental conditions. As some of the data are of an empirical nature, it would not be justifiable to assume *a priori* that results obtained with boxes of different shapes are directly comparable. In order to test the extent of the differences between the two types of cylindrical box involved in

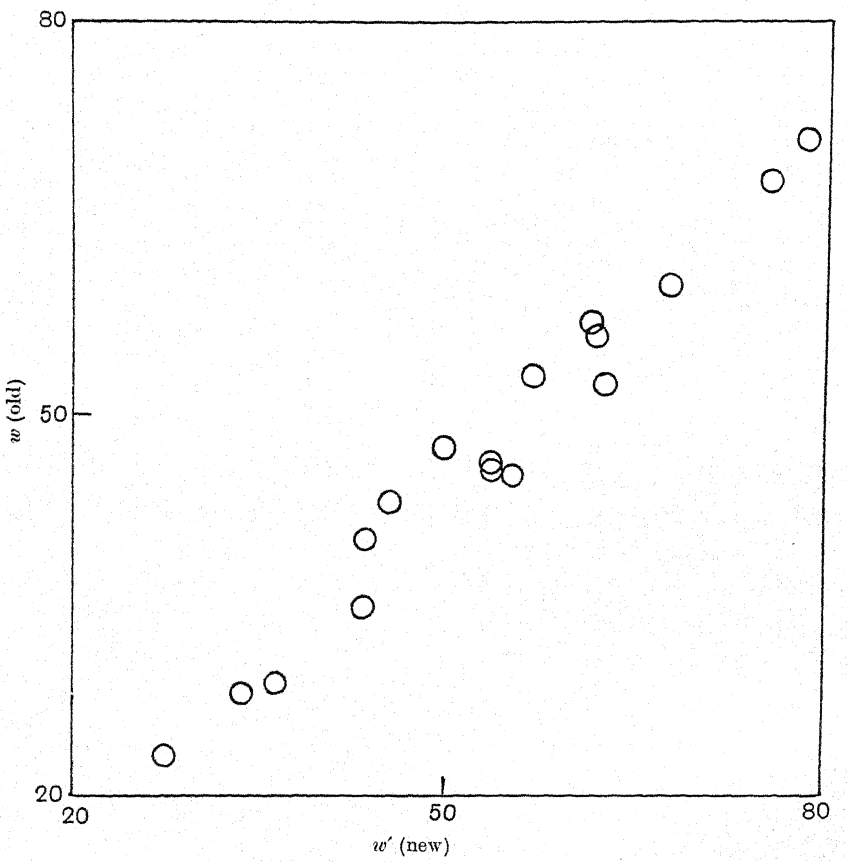


Fig. 1. Comparison of percentage moisture contents of saturated soils in old and new boxes.

the present discussion, experiments were performed with the new boxes on a number of soils from the series examined in detail in Paper II. Seventeen soils were selected, showing as wide a range of properties as possible. Particulars of their origin and chief physical characteristics will be found by reference to Paper II, Tables I and II, Numbers 8, 11, 16, 25, 26, 30, 34, 40, 41, 42, 46, 52, 57, 60, 62, 65, 66.

The data of chief interest obtained from the experiment are (*a*) the water content of the soil (expressed as a percentage of the air-dry soil) after attaining equilibrium by leaving the box to stand for a day with its perforated bottom in water; (*b*) the pore space of the air-dry soil; and (*c*) the swelling: the pore space and swelling are expressed as percentages of the volume originally occupied by the air-dry soil—*i.e.* the volume of the box. Comparisons of the results obtained with the seventeen soils are shown in Figs. 1–3, and numerical relationships between them have been calculated by the Cauchy-Awbery method (1).

(*a*) WATER CONTENT.

It will be seen from Fig. 1 that there is a close connection between the results obtained by the two methods. Let w and w' be the water contents obtained with the "old" and the "new" boxes respectively. Then if we assume that $w' = 0$ when $w = 0$, the relationship between the two is

$$w' = 1{\cdot}15w.$$

But taking the more general form, $w' = mw + c$, we obtain

$$w' = w + 6{\cdot}7,$$

and the line represented by this equation gives a more satisfactory fit than the other. The presence of a constant difference between w and w' is probably accounted for by the non-uniform distribution of water in the soil. As pointed out by Keen and Raczkowski (*loc. cit.*), the moisture content of the soil that rises above the top of the box is higher than that of the residual soil in the box. With the older boxes, the average difference for the seventeen soils under consideration was 18·0 per cent.; with the new boxes for the same soils it was 12·6 per cent., so that the difference in the former case is 5·4 per cent. greater than in the latter. Further, for a given soil, the water content of the portion swelling above the rim of the box was, approximately, the same for the different boxes. Hence, the final difference of, say, 6 per cent. is to be accounted for in some way by additional water held in the soil in the new box. A tentative explanation of the non-uniform moisture distribution will be mentioned later.

Results obtained with the old box showed that

$$w = S,$$

where S is the sticky point. In view of the further results, showing that the value of w depends to some extent upon the form of the box, the equality between w and S can only be regarded as fortuitous, but this

fact does not diminish the use suggested in Paper II of w (or w') as a soil constant, for the same set of correlations will hold in either case.

(*b*) PORE SPACE.

The results obtained for the pore space by the two methods (p and p' in Fig. 2) show very fair agreement: in judging the scattering of the

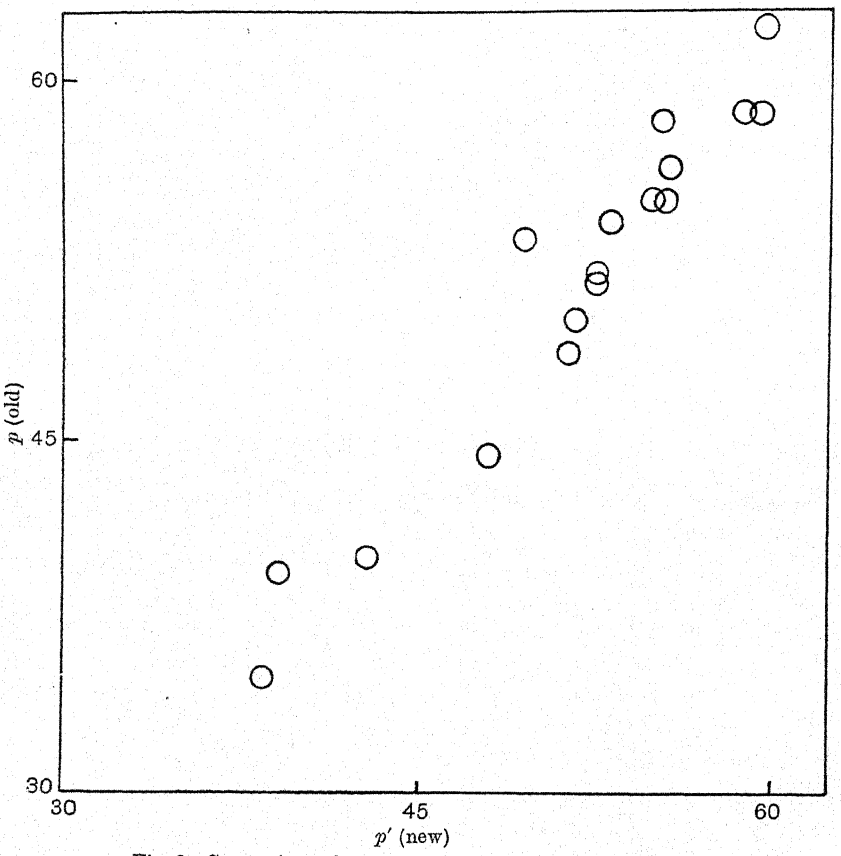


Fig. 2. Comparison of percentage pore spaces of air dry soils packed in old and new boxes.

points in the diagram, it should be remembered that a change of 1 per cent. in pore space is represented in Fig. 2 by the same length as a change of 2 per cent. in water content in Fig. 1. There cannot be any physical meaning to a constant term in the equation connecting p and p', and the data are satisfactorily expressed by the relationship

$$p' = 1{\cdot}02p.$$

The approximate agreement (to 2 per cent.) between the values of p and p' shows that the packing of the soil in the new box is practically the same as in the old.

(c) SWELLING.

Since the errors in the measurement of the swelling in replicate experiments under similar conditions are more serious than the corresponding errors in the measurements of the water retained and the pore space, it is not surprising that the scattering of the points representing the results for swelling obtained by the two methods (v and v' in Fig. 3) is

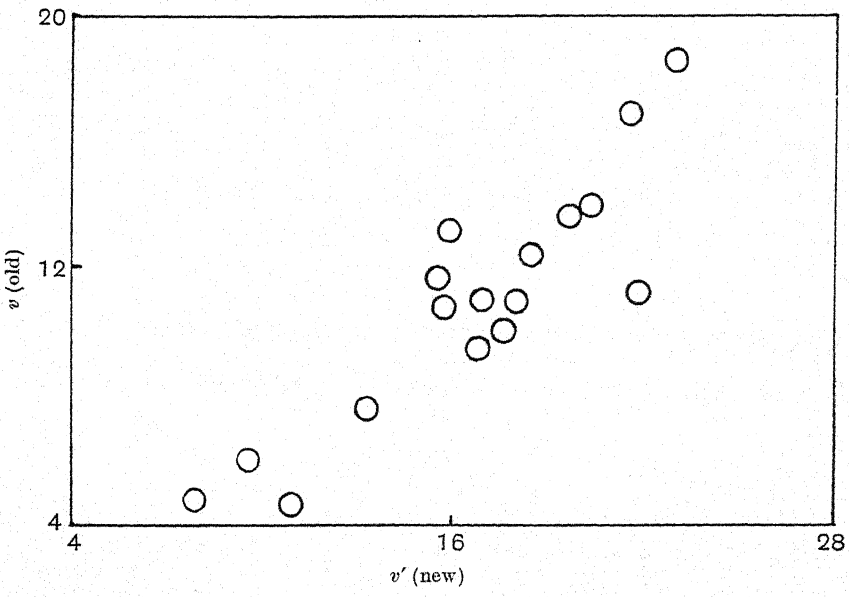


Fig. 3. Comparison of percentage swelling of soils packed in old and new boxes.

wider than in the cases of the other data. The most important feature of the results is that there is not approximate equality, or a constant difference, between v and v', but that they are connected by the equation

$$v' = 1{\cdot}56v.$$

The fact that the percentage swelling is greater with the shallower, new, boxes than with the deeper, old, boxes is not unexpected; actually the *absolute* expansions in the two cases are nearly equal: for the ratio of the volume of the old boxes to that of the new is 1·5—a figure in fairly close agreement with the coefficient of v' in the equation above.

Discussion of results.

The results quoted above suggest that most of the swelling takes place near the boundaries of the soil; the excess water content in the surface layers of soil confirms this view, for the swelling is evidently accompanied by imbibition of water. The "flooded" condition of the soil removed in the measurement of the volume swelling justifies the assumption that this soil is completely saturated, and a similar condition applies to the lowest strata at the bottom of the box, in contact with the free water below the filter paper. But the same state of affairs does not apply to the soil in the body of the box. When the water rises through the porous material, a film is formed between two adjacent soil particles, having a large curvature, concave outwards: consequently, the pressure inside the film is less than that outside, and the result is an apparent attraction between the particles. Such attractions, summed for the large number of contacts in the box, gives a large force tending to hold the particles together—*i.e.* to counteract the expansion due to imbibition. At the top and bottom of the box, where the water surfaces are plane and horizontal, the force vanishes; and in layers near the top of the box, where the packing of the soil is looser and the curvature comparatively large, the force is correspondingly smaller than in the body of the box. In the new boxes, it is possible that the introduction of the split ring has the effect of loosening the packing in its immediate vicinity (we have seen that the pore space calculated from the new boxes is slightly greater than that calculated from the old). If this is the case, the additional water held in the new box (about 6 per cent.) may be accounted for by an increase in thickness of the completely saturated layer at the bottom of the box.

It should be added that at no time during the experiment does the force due to capillary attraction vanish in the body of the box; the soil there is not fully saturated, since air bubbles are trapped in the pores as the water rises. The presence of such bubbles was recognised by Keen and Raczkowski (*loc. cit.*). It is also in accordance with Haines' analysis (4) of the changes taking place as water rises through an ideal soil: for Haines shows that it is possible for local saturation and a "funicular" distribution of water to co-exist. In another paper, Haines ((3), p. 285) refers to results obtained with sandy soils and sands by MacLaughlin and by Wadsworth and Smith, who examined the variation of moisture content with height in a column of which the base was immersed in water. MacLaughlin found a region of maximum moisture occurring above the

free water level. The conditions of his experiment were different from those holding in the case now under discussion, since strata of comparatively dry soil lay above the saturation level, but his results are obviously related to the present problem, and Haines' explanation of them, as being due to a loosening of the packing in the region of the saturated water front, is in general agreement with the views developed in the preceding paragraph.

A point of practical importance is that, since the absolute expansion is nearly as great with the new boxes as with the old boxes, the experimental errors in its determination are not increased by using a smaller quantity of soil. Advantages that can be claimed for the newer technique therefore include a considerable saving of time in the preliminary sieving of the soil sample, in the packing of the box, and in the subsequent oven drying.

SUMMARY.

A modified technique for the Keen-Raczkowski box experiment is described; it is believed that this new method is as accurate as, and is more convenient than, the one employed earlier.

The results obtained for pore space by the new method are very nearly the same as those obtained by the old method. The old and new results obtained for water retention and swelling are not the same, but are related to one another by simple linear expressions, and correlations with other soil properties deduced from the old data are applicable also to the new.

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(*Received March 29th*, 1930.)

"SINGLE VALUE" SOIL PROPERTIES. A STUDY OF THE SIGNIFICANCE OF CERTAIN SOIL CONSTANTS.

IV. A FURTHER NOTE ON THE TECHNIQUE OF THE "BOX" EXPERIMENT.

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(With One Text-figure.)

One of the previous papers by Coutts(1) in this series has directed attention to the value of the data obtained from what is now generally referred to as the perforated box experiment devised by the present writer and Raczkowski(3). In a further paper Coutts(2) has described an improved and simplified technique, which gives results that are either identical with the old method (pore space) or else related to them by simple linear expressions (water retention; volume expansion or "swelling"). As Coutts' procedure is now being followed at Rothamsted, and is likely to be taken up by other workers, it appears desirable to record some hitherto unpublished data bearing on the technique of the method, obtained at Rothamsted some years ago by Prof. Ohga (S.M. Educational Institute, Mukden, Manchuria) and dealing with the effect on the results of the degree of packing to which the soil is subjected. The original method, followed by Coutts in its essential details, is to add the soil in small quantities, tapping the box on the bench after each addition, until the box is nearly full, at which stage the filling is completed in the manner described in the original communication.

The procedure is empirical, but gives reasonably concordant replicate results in the hands of different workers; evidently the soil rapidly settles down to a certain apparent specific volume, and once this is attained the value is not appreciably altered by continuing the tapping on the bench.

Ohga examined the effect of consolidating the contents of the box by impact with a falling weight, after each addition of material. He also used precipitated silica instead of soil, to see whether the "swelling" with water was a property of soil *per se*, or whether another finely divided material would show the same effect. The silica was subjected to a mechanical analysis with the following result, in which the figures have been converted to the International scale:

Clay	Silt	Fine sand	Coarse sand
12·0	27·5	37·7	22·5

Hence, as far as its particle sizes are concerned, the material ranks as a medium to light loam.

The silica was first packed into the box in the usual way. The weight needed was only 26·2[1] gm. as compared with about three times this value for soil. Consolidation of the contents by allowing a weight to fall once on to the surface, and then filling the depression, increased the amount to 28·0 gm. In neither case did any swelling of the contents occur when the boxes were placed in water.

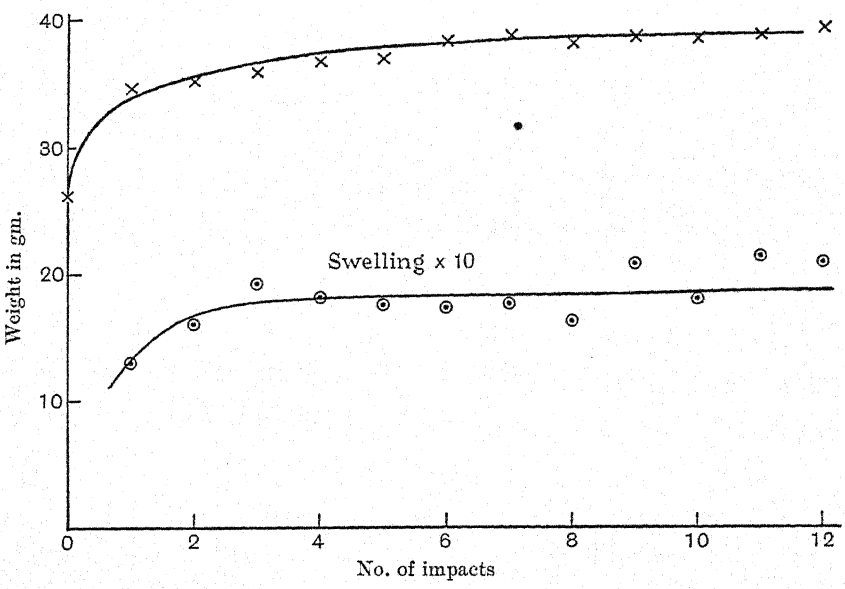


Fig. 1. Effect of number of impacts on weight of silica held by the box, and the volume expansion.

A series of experiments was then made, in which the silica was added to the box in small quantities, the contents being compressed after each addition. A cylindrical weight was employed, sliding within a vertical tube, and falling each time from the same height. The number of impacts for each addition of soil remained the same in each experiment. The results for the weight of silica in the box, and its volume expansion, or swelling, when placed in water are shown in Fig. 1.

Compression of the contents during the filling increases the weight from 26·2 gm. to nearly 40 gm., but there is little increase for the higher

[1] The old type of box of square cross-section was employed, which had a volume about 27 per cent. greater than the standard cylindrical pattern.

number of impacts and, as would perhaps be expected, the single impact is proportionally the most effective. The volume expansion is subject to an appreciable experimental error, but it appears to arrive at its maximum value before the other curve.

These results are, of course, preliminary. They suggest that like the weight per unit volume, the swelling is a function of the degree of packing to which the material is subjected during the filling of the box. Further, the fact that such inert material as precipitated silica can show a "swelling" when saturated with moisture raises the question as to how far imbibitional moisture is concerned in the volume expansion of soil. These points are being put under investigation.

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(*Received April 15th*, 1930.)

STUDIES IN CROP VARIATION.

VII. THE INFLUENCE OF RAINFALL ON THE YIELD OF BARLEY AT ROTHAMSTED.

COMPLETED BY

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FROM AN INVESTIGATION INITIATED BY

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(With Seven Text-figures.)

I. Introduction.

An earlier study by W. A. Mackenzie(1) consisted of an examination of the yield of dressed grain from Hoos Field, where barley has been grown continuously under different manurial treatments since 1852. Thirteen plots, the treatment of which has, with minor exceptions, remained constant over the whole period of the experiment, were selected for detailed statistical analysis. These plots were: the five plots of the "O" series (see Table I), the five plots of the "A" series, the unmanured plots 6–1 and 6–2, and the farmyard manure plot 7–2. The method used, following that of R. A. Fisher in his study of the wheat yields from Broadbalk(2), was to fit a polynomial curve up to the fifth degree to each of the series of yields. It was found that when classified according to the significance of the terms in the fitted polynomial curves, the groups were the same as when arranged according to manurial treatment. These groups were:

(1) "O" series, 6–1, 6–2. No nitrogenous manure. Heavy deterioration of yield from beginning. Rate of decline arrested slightly between 1870 and 1880. Lowest point reached about 1900, after which a rise to about 1914, since when the yield has fallen.

(2) 1–A and 3–A. Nitrogenous manure but no phosphoric manure. Deterioration similar to (1) but at a higher level of yield. Fall completely arrested between 1860 and 1875.

(3) 2–A, 4–A, 5–A and 7–2. Nitrogenous and phosphoric manures. Yield increased for first ten years or so (sixteen years in case of dunged plot). Deterioration arrested about 1895 except for 7–2, but 5–A was the only plot to show a rise at the beginning of this century.

Table I.

Plot		Manures per acre: Farmyard manure (tons)	Rape cake (lb.)	Ammonium salts (lb.)	Nitrate of soda (lb.)	Super-phosphate (cwt.)	Sulphate of potash (lb.)	Sulphate of soda (lb.)	Sulphate of magnesia (lb.)	Silicate of soda (lb.)	Mean yield (1868–1916) per acre and standard error of mean (*bushels*)
1–0	...	—	—	—	—	—	—	—	—	—	13·97 ±0·76
2–0	...	—	—	—	—	3·5	—	—	—	—	19·62 ±0·91
3–0	...	—	—	—	—	—	200	100	100	—	15·00 ±0·87
4–0	...	—	—	—	—	3·5	200	100	100	—	19·85 ±1·06
5–0	...	—	—	—	—	3·5	200	—	—	—	16·22 ±0·97
1–A	...	—	—	200	—	—	—	—	—	—	24·90 ±1·10
2–A	...	—	—	200	—	3·5	—	—	—	—	37·11 ±1·51
3–A	...	—	—	200	—	—	200	100	100	—	27·13 ±1·15
4–A	...	—	—	200	—	3·5	200	100	100	—	40·75 ±1·14
5–A	...	—	—	200	—	3·5	200	—	—	—	34·92 ±1·22
1–AA	...	—	—	—	275	—	—	—	—	—	25·52 ±1·14
2–AA	...	—	—	—	275	3·5	—	—	—	—	40·12 ±1·24
3–AA	...	—	—	—	275	—	200	100	100	—	26·14 ±1·12
4–AA	...	—	—	—	275	3·5	200	100	100	—	39·31 ±1·22
1–AAS	...	—	—	—	275	—	—	—	—	400	31·79 ±1·17
2–AAS	...	—	—	—	275	3·5	—	—	—	400	41·15 ±1·33
3–AAS	...	—	—	—	275	—	200	100	100	400	33·16 ±1·28
4–AAS	...	—	—	—	275	3·5	200	100	100	400	41·63 ±1·27
1–C	...	—	1000	—	—	—	—	—	—	—	38·38 ±1·14
2–C	...	—	1000	—	—	3·5	—	—	—	—	40·56 ±1·18
3–C	...	—	1000	—	—	—	200	100	100	—	37·00 ±1·20
4–C	...	—	1000	—	—	3·5	200	100	100	—	40·57 ±1·21
6–1	...	—	—	—	—	—	—	—	—	—	15·51 ±0·85
6–2	...	20 bushels ashes from laboratory furnace									16·46 ±0·83
7–1	...	—	—	—	—	—	—	—	—	—	22·46 ±1·56
7–2	...	14	—	—	—	—	—	—	—	—	46·17 ±1·20

The main conclusions from the study were: (1) a comparison of certain plots showed an apparently adverse influence of sulphate of potash; (2) the very great importance of superphosphate to the barley crop was confirmed—this fertiliser not only gave a greatly increased yield when applied, but maintained the fertility of the soil to such an extent that the annual deterioration was found to be least on the plots containing superphosphate; (3) the deterioration of the barley plots has been much heavier than that of the wheat plots; and (4) barley has been more variable than wheat and more subject to the influence of meteorological conditions.

In the present paper a study is made of the influence of rainfall on the barley of the Hoos Field plots, the method used being that devised by R. A. Fisher in his study of the wheat plots on Broadbalk(3).

II. DESCRIPTION OF METHOD.

The yields for the 70 years, 1852–1921, were used in the earlier study, but owing to the fact that the rainfall records at Rothamsted with the large gauge of 1/1000th acre began in February 1853, there were only available 68 complete harvest years, 1854–1921. (The harvest year is taken as the period September 1–August 31.) It was necessary, therefore, to leave out of account the yields for the first two years. 1921 was the last year available when the investigation was begun and, although some years have elapsed since then, the later figures could not have been used without repeating a large amount of work that had already been performed; as they stand, too, the results will be more comparable with those of the earlier study. As the field was fallowed in 1912 the figures for yield for this year were estimated as the averages of the three preceding and three following years, for the purpose of eliminating secular trend, while the yields for 1913 were very much heavier than usual, following the fallow. Because of this it was decided to leave these years out of account in calculating the rainfall regressions.

For the general method recourse should be had to R. A. Fisher's wheat study(3). It may be instructive, however, to give in order the successive stages in the arithmetical handling of the data. First the rainfall distribution values were calculated for the years 1919, 1920 and 1921 by dividing the year's rainfall up into 61 periods of six days each and analysing as described by Fisher ((3) pp. 111–12). The following values were obtained, and are supplementary to Fisher's Table IV, p. 114.

Table II. *Distribution values of rainfall.*

Harvest year	a'	b'	c'	d'	e'	f'
1919	592	−52	+19	+22	+51	+10
1920	481	+19	−30	+13	−25	−26
1921	267	−56	− 5	+14	+ 3	+ 1

The rainfall of each year is specified by six distribution constants. a' is the average amount of rain in thousandths of an inch, falling in a six-day period, b' is proportional to the average rate of change of this amount from one period to the next, and so on. These quantities a'–f' are then regarded as the independent variates with which the crop yields, y, on the different plots are to be successively correlated. As, however, the yields, and to a smaller extent the rainfall distribution values, are subject to secular trends, it becomes necessary to eliminate these trends by correlating, not the actual values, but the residuals left after fitting polynominals up to a degree necessary to take account of all trend. This was what was actually done, polynomials being fitted to the fifth degree in all cases. But it was not necessary to calculate the actual polynomials, save for a few years at the end of the series. The 68 values of a' were analysed by summation as described by Fisher ((3) p. 113), the successive sums S_1–S_6 being very easily obtained from the old series by adding in the three new values. From these sums a series of numbers a–f was calculated, then the series a'–f', and finally the quantities $x_2' \ldots x_6'$. (See Fisher(3), p. 115.) Then it is only necessary to calculate the sum of squares of the 68 deviations of the a''s from their mean 468·54, and to subtract the sum of the squares of the five x''s. The result is the same as if we had calculated the 68 polynomial values determined by fitting a curve of the fifth degree, found the difference: observed–calculated, and summed the squares of these differences. The labour of the calculation of the polynomial values is, however, avoided by using the first method. Since it was decided to leave out the figures for 1912 and 1913, it was necessary to calculate the polynomial values for these years. This was conveniently done, as these years were nearly at the end of the series, by the method ((4), pp. 129–31) of calculating the terminal polynomial value and the backward differences. The sum of the squares of the two differences (observed–calculated), for the years 1912 and 1913, was then subtracted from the previous total, and the remainder, representing the sum of squared deviations of the a' rainfall coefficients from their polynomial values, but leaving out 1912 and 1913, formed one element in the matrix of squares and products of rainfall deviations.

This procedure was followed for the other rainfall values b'–f', and the following table gives the values in each case, together with the difference (actual–polynomial) for the years 1912 and 1913.

Table III. *Secular changes in rainfall constants.*

	a'	b'	c'	d'	e'	f'
x_2'	+113·04	−28·61	−47·65	+85·34	+16·93	− 5·38
x_3'	− 67·10	+39·88	−42·51	− 4·31	− 4·25	− 4·56
x_4'	+105·48	−54·01	−34·40	+ 0·20	+27·29	−24·24
x_5'	− 60·33	− 1·42	+39·25	− 9·67	−17·92	+ 4·25
x_6'	−208·66	+ 3·91	− 9·57	−30·68	+22·07	+19·56
1912	+152·81	+29·13	+27·79	+80·43	− 1·06	+ 1·42
1913	− 70·47	−42·04	−39·04	− 9·16	+ 5·74	+22·24

From this table was calculated the sum of squares of the figures in each column, and also the sum of products of figures in adjoining columns, two at a time. All it was necessary to do then was to calculate the crude sums of squares and products of the rainfall values from Fisher's Table IV ((3), p. 114) supplemented by our Table II, and to subtract the appropriate correction. The result is shown in Table IV below. The number of degrees of freedom is in each case 60, since five are accounted for by the fitted terms of the polynomial and two for the two years left out of account.

Table IV. *Sums of squares and products of deviations.*

	a'	b'	c'	d'	e'	f'
a'	+411,547	+ 3,946	+35,221	+29,248	− 2,986	+ 8,443
b'	+ 3,946	+150,538	+ 3,983	− 8,237	−16,261	− 8,229
c'	+ 35,221	+ 3,983	+54,325	− 4,054	+10,646	+ 3,562
d'	+ 29,248	− 8,237	− 4,054	+39,826	− 6,669	+ 7,279
e'	− 2,986	− 16,261	+10,646	− 6,669	+29,170	+ 44
f'	+ 8,443	− 8,229	+ 3,562	+ 7,279	+ 44	+23,887

This table is to be used in conjunction with the actual yields of barley to determine partial regressions, for 13 separate plots. It is, therefore, advisable to form the matrix of multipliers, each of which is the co-factor of the number above, regarding Table IV as a determinant, divided by the value of the determinant itself. The method used was that of solving successive series of linear equations ((4), pp. 133–4). The following table shows the results in millionths.

Table V. *Co-factors for determining partial regressions.*

	a'	b'	c'	d'	e'	f'
a'	+2·763525	−0·087217	− 2·038787	− 2·166214	+ 0·483168	− 0·043597
b'	−0·087217	+7·465890	− 1·501184	+ 1·906227	+ 5·133296	+ 2·236324
c'	−2·038787	−1·501184	+21·948851	+ 2·719268	− 8·428536	− 3·882629
d'	−2·166214	+1·906227	+ 2·719268	+29·981892	+ 6·715343	− 8·131785
e'	+0·483168	+5·133296	− 8·428536	+ 6·715343	+41·803151	+ 0·731135
f'	−0·043597	+2·236324	− 3·882629	− 8·131785	+ 0·731135	+45·705270

We now turn our attention to the series of plot yields. For each plot a polynomial of the fifth degree was fitted, in the earlier study, by the method of successive summation. Seventy years were available, and the first 34 of these were summed from the beginning, the remaining 36 being summed backwards from the terminal value, dropping one term at the end of each summation. To adjust the sums S_1, S_2 ... S_6 for the omission of the first two years, it was necessary to subtract from the sums of the upper series the quantities

$$a + b, \quad na + (n-1)\,b, \quad \tfrac{1}{2}n\,(n+1)\,a + \tfrac{1}{2}\,(n-1)\,nb, \quad \text{etc.},$$

where a and b are the yields for 1852 and 1853 respectively, and n is 34. From the broken sums a new series S_1, S_2 ... S_6 was formed as described by Fisher ((3), p. 111), noting that the lower series now consists of 36 quantities, and not 32. From these, new polynomial coefficients were calculated, and hence a new series of quantities x_2' ... x_6', one for each plot. Each plot is now to be regarded as the dependent variate in turn. Just as in the case of the independent variates, the rainfall coefficients, polynomial values for the years 1912 and 1913 were calculated and the difference (actual–polynomial) found. We give below (Table VI) the values of these important quantities for the thirteen plots studied.

Table VI. *Secular changes in yield series.*

	1–0	2–0	3–0	4–0	5–0	1–A	2–A
x_2'	−27·4742	−22·5046	−32·9699	−28·6486	−31·0042	−40·1574	−61·3729
x_3'	+16·7833	+23·8117	+25·0033	+30·1629	+15·6200	+14·7588	+10·7268
x_4'	− 3·6909	− 5·8102	− 2·0517	− 5·1083	− 6·7681	− 0·3469	+ 7·0273
x_5'	+ 6·9657	+ 0·7007	+ 2·9708	− 0·4588	− 1·8157	− 2·4166	− 8·5642
x_6'	− 9·3378	−11·6279	−11·7244	−10·7766	− 7·1338	−22·5894	− 5·2337
1912	− 1·2128	+ 0·2277	− 0·2274	− 0·3692	− 0·1473	− 0·4066	− 0·7988
1913	+ 8·6730	+14·8186	+11·9700	+20·8982	+16·8044	+15·3168	+31·1862

	3–A	4–A	5–A	6–1	6–2	7–2
x_2'	−47·3378	−34·3690	−51·1542	−29·6203	−25·9940	−29·6965
x_3'	+ 9·4928	+ 6·8426	+19·4076	+20·8670	+14·9025	−13·2157
x_4'	+ 0·1055	− 5·9576	+10·5437	− 5·5448	− 6·8142	+ 4·0662
x_5'	− 4·6413	− 6·4123	−10·8570	+10·2833	+ 4·3100	− 2·5434
x_6'	−20·1451	− 7·4670	−12·1215	−11·0210	−13·0517	− 4·9726
1912	+ 0·6615	+ 2·0562	− 0·8466	− 0·4664	− 0·9039	− 1·0273
1913	+11·1684	+22·6721	+17·1289	+10·6702	+ 8·1500	+19·9137

For each plot the sums of products of (*a*) deviations of actual yield values from the mean of the 68 years, and (*b*) deviations of the rainfall distribution values from their means, were calculated, there being six such sums for each plot. From each sum was deducted the sum of products of the column of figures in Table VI for that plot and the appropriate column of Table III. We had then six corrected sums of products, which were subsequently multiplied by the values of any column of

Table V and added, thus giving the regression of yield for this plot on the corresponding rain variate. With rainfall measured in inches and the crop in bushels per acre, the values obtained for the regression coefficients are given in Table VII below:

Table VII. *Partial regressions of barley yields on*

Plot	a'	b'	c'	d'	e'	f'
1–O	−22·0846	+ 2·8805	+27·9547	+15·8658	+ 4·8543	− 20·5088
2–O	−20·5280	+10·2283	+25·2228	− 9·4099	−10·8412	+ 0·6026
3–O	−18·9447	− 2·6663	+28·2998	+17·5088	− 6·3732	− 35·7710
4–O	−17·8957	+10·6243	+21·3957	+ 1·1587	− 8·2207	− 23·3711
5–O	−16·6183	− 8·5029	+31·0215	+25·6261	−24·2634	− 26·6001
1–A	− 6·9219	−18·3234	+19·2871	− 8·1509	−54·9271	− 72·0898
2–A	−27·0151	+ 8·8218	+29·6595	−17·8824	−50·7409	− 10·5889
3–A	−12·8891	−37·9575	+36·7535	+29·0490	−42·4456	−108·0633
4–A	−18·2951	− 9·8460	+21·4513	− 7·6631	−55·9328	− 60·2210
5–A	−19·1056	− 9·1185	+31·2658	+30·8624	−29·6743	− 65·3984
6–1	−17·6500	+ 8·1595	+31·4329	+12·7247	−20·3209	− 45·7877
6–2	−21·5369	+ 8·0100	+36·3300	− 0·8196	−30·9715	− 33·2914
7–2	−24·9369	− 3·5280	+71·0287	−67·8273	−74·7612	− 13·1281

The coefficients of the above table give linear regression formulae to express the deviation of the barley crop on each plot in terms of the rainfall distribution values. In order to obtain curves analogous to those of Fisher for the wheat crop, showing the average benefit or loss in bushels per acre ascribable to an additional inch of rain over the average at any time during the year, the six regressions are to be divided by the factors

$$\frac{(r\,!)^2}{(2r)\,!}\,61\,.\,62\,\ldots\,(61+r)$$

for $r = 0, 1, 2, 3, 4$ and 5. We then have the coefficients A, B, C, etc., in the expansion of the average rainfall effect in a series of orthogonal polynomials

$$a = A + Bt + C\,(t^2 - n_2) + \ldots$$

(see Fisher (3), pp. 97–9), where t is the time in six-day intervals measured from the central period of the year, *i.e.* t runs from -30 to $+30$. n_2 is the mean value of t^2 and is equal to $\frac{1}{12}(n^2 - 1)$, where $n = 61$. The other mean values, those of t^4, t^6, etc., are stated by Fisher ((3), p. 99) and the above expression can be readily calculated for each plot, the computation being facilitated by the initial preparation of tables showing for different values of t from -30 to $+30$ the values of the polynomial expressions in t that enter into the above equation. The course of the function a throughout the year is shown in the diagrams at the end of this paper.

III. Analysis of curves.

The curves show the average benefit, or loss, in bushels per acre to be expected from an additional inch of rain over the average, at any time during the year. Thus a point on the zero line would indicate that crop yield was independent of the rain falling at that period; where the curve is above the zero line excess of rain is beneficial to the barley crop, and where it is below the line excess of rain is detrimental. An examination of the curves reveals the following general features: (1) there is in most cases a period of maximum damage or minimum benefit, centred about October; (2) the damage is at its minimum in the winter months with the exception of 2–0, 2–A and 7–2, the curves for which are rather different in character from those of the other plots; (3) in the case of certain plots of the A series, namely the plots with nitrogenous manure but without phosphate, excess of rain is beneficial during autumn and winter, the benefit being greatest in December; (4) a further period of maximum damage, much greater than in autumn, is centred about the period March–April; (5) with practically all the plots excess of rain is beneficial to the crop in the summer months.

The form of the curves suggests that the plots may be classified in the same general groups as in the previous study, where the grouping was according to the significance of the polynomial terms fitted (see p. 417). Plots 6–1 and 6–2 are adjacent, and are alike in that neither receives a fertiliser dressing. The only difference between them is that plot 6–2 receives 20 bushels of ashes from the laboratory furnace. This does not appear to have affected the rainfall response; there is a mean difference in yield of 1 bushel per acre, but this difference is only of the order of its standard error (which was 1·19 bushels over the period 1868–1916) and cannot be judged significant. A comparison of the rainfall curves for 6–1 and 6–2 will therefore give some idea of the order of difference to be expected in the other curves for significance. They are shown in Fig. 1. Two plots, however, stand out from the classification adopted, namely 2–0 and 2–A, and these are the plots which have already been noted as showing a pronounced phosphate effect. The farmyard manure plot 7–2 presents some little difficulty, just as it did in the previous study, and in view of the very different character of the treatment it receives from that of the others it may require to be considered as falling in a group by itself. The nitrogenous dressing is very much heavier than on the plots of the "A" series, so that heavier damage due to rain is to be expected at certain seasons of the year; the fact, too, that

the dressing of farmyard manure has a pronounced residual effect on following crops, while drainage is very different owing to the different texture of the soil, renders it unlikely that the rainfall curve should be at all like those of other plots. The curve is shown in Fig. 7.

Group 1. *No added nitrogen.*

(*a*) Plots 1–0, 6–1, 6–2 (unmanured), 3–0 (alkali salts), 4–0 (alkali salts and phosphate) and 5–0 (potash only and phosphate). The curves are shown on Figs. 1, 2, 3 and 6.

All these plots are very similar in their rainfall curves. The mean yields per acre for the period 1854–1921 are as follows:

Plot	Yield in bushels	Plot	Yield in bushels
1–0	13·67	3–0	14·65
6–1	15·15	4–0	19·43
6–2	16·17	5–0	15·91

The curves are best described numerically by stating the benefit or loss in bushels per acre due to an additional inch of rain for various periods of the year where the curves reach maximum and minimum values, or remain stationary for some time. The amounts at these times, together with those at the starting points (average September values), are set out in the following table. A negative sign shows loss and a positive sign benefit, due to excess of rain:

1–0	−0·15 (September)	−0·37 (end October)	−0·32 (mid December)
6–1	−0·17 („)	−0·35 (mid „)	−0·18 (mid „)
6–2	−0·13 („)	−0·29 (end „)	—
3–0	−0·06 („)	−0·27 (mid „)	−0·15 (early „)
4–0	−0·20 („)	−0·40 (end „)	−0·34 (mid „)
5–0	−0·06 („)	−0·06 (September)	+0·06 (mid November)

1–0	−0·69 (end April)	+0·16 (end August)
6–1	−0·78 (early „)	+0·35 (end July)
6–2	−0·84 (end March)	+0·27 (end „)
3–0	−0·76 (mid April)	+0·10 (early August)
4–0	−0·54 (end March)	+0·15 (end July)
5–0	−0·80 (mid April)	+0·10 (early August)

There is only one significant point of difference about the curves and that concerns 5–0, the curve for which remains remarkably close to the zero line right up to the middle of December. Over this period excess of rain has not proved harmful, nor has it improved the yield. This is the only portion of any of the curves that seems to show the crop yield quite independent of the amount of rain falling at this time.

(*b*) Plot 2–0 (phosphate only). This curve is shown in Fig. 3. The mean yield of plot 2–0 is 19·28 bushels. Beginning at − 0·16 in September, the loss increases steadily until the minimum point of the curve

is reached at the beginning of February, the value at this point being − 0·62. The curve then turns upward until it reaches a maximum coinciding with the zero line at the end of July and beginning of August. The curve is the steadiest of the whole series, and is little affected by exceptional conditions obtaining at the time of sowing. Excess of rain is nowhere beneficial.

Group 2. *Nitrogenous manure but no phosphoric manure.*

Plots 1–A (nitrogen only) and 3–A (nitrogen and alkali salts). The curves are shown in Fig. 4. They are alike, but 3–A shows the same effects in a more exaggerated form, while both show distinct points of difference from Group 3, which contains the remaining plots of the "A" series. Mean yields (1854–1921) are: 1–A, 24·52 bushels; 3–A, 26·86 bushels. The curve for 1–A shows a benefit to the crop through excess of rain extending right up to the middle of January, while 3–A reaches the zero line a week or two later. The average values for September and the maxima and minima are given in the following table:

1–A	+0·32 (September)	+0·08 (early October)	+0·26 (early December)
3–A	+0·48 („)	+0·10 (early „)	+0·55 (mid „)

1–A	−0·74 (early April)	+0·43 (mid July)
3–A	−1·31 (mid „)	+0·28 (mid „)

Thus while 3–A shows a more pronounced benefit over the autumn and winter period, this is offset by a greater loss over the spring and early summer, and the period during which excess of rain benefits the crop towards the end of growth is reduced by some three weeks. The maximum loss on 1–A in early April is of the same order as the maximum loss on 1–O, although in the case of 1–O this point is only reached at the end of the month. The difference is only 0·05 bushel, which is not significant. Comparing 3–O and 3–A, however, we find in mid-April that the loss on 3–A exceeds that on 3–O by 0·55 bushel, a quite significant amount. In contrast to the plots of the O series, the curves turn down sharply during August, the average value over this month being − 0·56 bushel for both plots.

Group 3. *Nitrogenous and phosphoric manure.*

(*a*) Plot 2–A (nitrogen and phosphate only). This curve, being different in character from the others, is treated separately. The curve is shown in Fig. 5. The average yield is 37·04 bushels. Beginning with an average value of − 0·28 in September, an early maximum of − 0·21

is reached at the beginning of October. From this point on the loss becomes progressively greater until a minimum point of − 0·94 is reached in late February. After this the loss diminishes, the curve crossing the zero line in the middle of June, and reaching a maximum of + 0·16 in the first weeks of July. After the end of this month there is an increasing loss, the average value over August being − 0·36. This curve reflects the same general features as 2–O, but the loss is greater during the winter and spring months.

(*b*) Plots 4–A (nitrogen, alkali salts and phosphate) and 5–A (nitrogen, phosphate and potash only). These plots are very similar in their rainfall response. The curves are shown in Figs. 5 and 6. The average yields are: 4–A 40·79 bushels; 5–A 34·82 bushels. In comparing these curves it should be noted that there was no corresponding similarity over the autumn and winter period between plots 4–O and 5–O, in which nitrogen is omitted. In fact 4–A and 5–A behave like 5–O in showing themselves very nearly independent of the rainfall up to December. The starting and final values of the curves, with the maxima and minima, are as follows:

4–A	− 0·01 (September)	− 0·13 (early October)	− 0·01 (end November)
5–A	− 0·11 („)	− 0·24 (early „)	+ 0·11 (early December)
4–A	− 0·88 (end March)	+ 0·30 (mid July)	− 0·59 (August)
5–A	− 1·06 (mid April)	+ 0·25 (end „)	− 0·04 („)

The points of difference are these: 5–A shows a slight positive benefit in December, while 4–A does not rise above the zero line; 4–A reaches a minimum earlier, and the loss at this time is not so great as with 5–A—excess of rain becomes beneficial to 4–A some three weeks earlier than in the case of 5–A—and finally the loss is greater on 4–A than on 5–A in August. The similarity of 5–O and 5–A is quite striking, the only difference being that 5–O is slightly more flattened out, *i.e.* the effect of excess of rain is not so marked except over the final period of July and early August, at which time the general tendency is for excess of rain to benefit plots of the "A" series more than those of the "O" series.

(*c*) Plot 7–2 (farmyard manure). The rainfall response curve (Fig. 7) for this plot is rather different in character from the others. It resembles most the plots having phosphate but no alkali salts (2–O and 2–A) but, on a more exaggerated scale. There is a difference, however, at the beginning, where for the first two months of the harvest year rain above the average is beneficial to the crop. This is the highest yielding plot on Hoos Field, and until recent years its fertility was maintained at a high level. The earlier study (1) showed that the deterioration was very much smaller

on this plot than on any of the others, the yield only falling off slightly from the 'sixties of last century until 1921. The decline has been much more rapid of recent years, however. The average yield over the period 1854–1921 was 46·51 bushels, but in spite of the fallow of 1912 the yield has continued to fall, the average of the years 1914–28 being 33·37 bushels. This fall has been common to nearly all the plots. The rainfall curve for 7–2 begins with an average value of + 0·73 over September. The curve falls steadily, passing through the zero line towards the end of October. From this point on until the latter half of May rain above the average reduces the yield, the lowest point on the curve being − 1·41 in mid-February. From the end of May until the beginning of August excess of rain is beneficial, the value reaching a maximum of + 0·50 at the beginning of July. Throughout August the curve falls rapidly in common with all the plots of the "A" series, the average for August being − 0·53.

IV. Comparison with Hooker's conclusions.

R. H. Hooker (5, 6) examined the influence of rainfall and temperature on a number of crops over the area of Eastern England. In his later study he had 35 years' data available, and his method was to determine correlation coefficients, total and partial, between crop yield, rainfall and temperature. The correlation coefficients were calculated for overlapping eight-week periods before and during the growing season. He found that the only significant correlations with rainfall in the case of barley were for the period from mid-December to the end of February, and were negative (− 0·45 and − 0·48). Positive correlations of smaller magnitude (0·38 and 0·36) were found for the period May–early July, and while he hesitated to regard these as significant, since they were below his critical value 0·5, he thought that they showed evidence of dependence. As a matter of fact the 5 per cent. point for the correlation coefficient for 35 years' data is 0·334, and the 1 per cent. point 0·430 (4), so that Hooker's figures of 0·36 and 0·38 seem to have a fair measure of significance, and he might well have been satisfied with a lower critical level than 0·5. Now the plot on Hoos Field which may perhaps be taken to resemble most closely the barley yields under commercial conditions of Hooker's investigation is the farmyard manure plot, 7–2, and there we find that the period of maximum damage caused by excess of rainfall is centred about February, while there is a period of benefit during June and July. With the other curves the minimum occurs significantly later, in March or April, with the exception of the plots having phosphate but no alkali salts, and the

period of benefit, where it occurs at all, is advanced to July. Hooker also determined partial correlations between barley yield and rainfall for constant temperature. The significant negative values over the winter period were not altered (the partial correlations being $-0{\cdot}46$ and $-0{\cdot}48$), showing that the effect is not due to associated higher temperatures at this time. The positive correlations for the summer period were reduced to about half their former values ($+0{\cdot}14$ and $+0{\cdot}25$). Thus while part of the rainfall effect at this time is undoubtedly due to the lower temperature associated with rainfall in summer, there is some evidence from Hooker's researches to show that there is also a moderate rainfall effect which is independent of temperature.

V. Comparison with wheat results from Broadbalk.

Although the crop is different, and likewise the field, our results invite comparison with R. A. Fisher's results for the influence of rainfall on the wheat plots from Broadbalk. The conclusion was there reached that secular changes were observable in the rainfall data and had to be taken account of in correlating these with the crop yields. Thus there have been two periods of very wet years since the Rothamsted records started, one centred about 1879 and another centred about 1914. Fisher observed that these periods agreed with the two main depressions in yield, but on further examination decided that the depression was probably not due directly to the heavy rainfall, but occurred because the sequence of wet years produced unfavourable conditions by fostering weeds. We cannot say the same about the barley plots in Hoos Field. Weeds here do not seem to be the cause of the slow changes, although at times the foulness of the land has been commented on, and in 1912 the whole field was fallowed on account of weeds. It so happens, however, that 1912 coincides with a secondary peak in yield in the case of most of the plots, the yield rising steadily from the beginning of the century. The sequence of wet years prior to 1914 does not, therefore, seem to have affected the yields of those years adversely, although the yields have in most cases fallen very rapidly since, while it is equally remarkable that the slowing down, and in two cases the complete arrest, of the deterioration in the 'seventies occurred just prior to the other series of wet years. These facts seem to indicate that wheat and barley react in a different way to rainfall above or below the average. This is a reasonable supposition, for wheat is in the ground for most of the year, while barley is not sown until the spring, and the influence of the important winter rainfall must therefore in this latter case be indirect,

influencing the texture of the land or the cultivation operations, or affecting the nutrients and micro-organisms present in the soil.

It may be recalled that the general nature of the curves showed that rain above the average was not only not beneficial, but was actually detrimental to the crop at whatever time it occurred. The exceptions were the period of about one month round about October, where the plots with a heavy nitrogenous dressing showed a slight benefit (plots 8, 10, 11, 12 and 14), while in two cases (plots 10 and 11) there was a further advantage in excess of rain in April. The main features of the curves were (1) a period of only slight damage, or even positive benefit, in autumn, (2) heavy damage during the winter months, ascribed to the washing out of soluble nitrates from the soil, (3) a further period of minimum damage (benefit in the case of plots 10 and 11) for the period April–May, (4) a further loss in summer, which becomes progressively greater the more unbalanced the application of fertiliser becomes, and finally, (5) a rapid reduction of this loss in August, probably due to the fact that harvest generally takes place then, and rain may occur in this month which is too late to affect the crop. These findings, which show that excess of rain was beneficial only on a few plots and over a very limited period, are in marked contrast to the results for barley. Thus it has been shown that with some of the barley plots excess of rain is beneficial over the whole of the autumn and winter, and that plots 2–O, 2–A and 7–2 are the only ones to show a significant loss over this period, while 7–2 is benefited considerably by rainfall above the average in early autumn. If we compare this curve with that of the farmyard manure plot (2 *b*) of Broadbalk, we see that it is similar in character over the winter months, although the loss in the case of the wheat plots is not so great, the minimum of $-1{\cdot}00$ bushels occurring at the beginning of January, as compared with a minimum of $-1{\cdot}41$ bushels on the barley plot 7–2 in mid-February. The wheat curve never rises to the zero line, while the barley curve shows a pronounced benefit due to excess of rain in June and July. The wheat and barley curves for the farmyard manure plots are similar in their steady fall over the last month of the harvest year, a feature which contrasts with the behaviour of all the other wheat plots, which turn up at the ends. There is a difference in the time of application of the farmyard manure, as the barley plots receive their dressing just prior to the sowing of the crop in March, while the dung goes on to the wheat plots at the end of September or beginning of October. The barley plots all contrast with the wheat plots in showing a benefit due to excess of rain over the summer period.

VI. SUMMARY.

The conclusions of an earlier study of the yield of dressed grain from Hoos Field over the period 1852–1921 are recalled, and the method of conducting an examination of the influence of rainfall on the yield is described. The method yields a series of curves showing at any given period of the year the benefit or loss to the final yield in bushels per acre due to an additional inch of rain over the average at that period. Thirteen plots are considered, and the curves are found to fall roughly into groups corresponding to the system of manuring adopted. The main effects noted are these: (1) Excess of rain is beneficial to the barley crop for a short period in summer, and, in the case of certain plots, over the autumn and winter period. This is in contrast to the corresponding result for wheat on Broadbalk, where excess of rain was found to be beneficial only on a few plots and over a very limited period. (2) Rainfall over the six months when the barley is not in the ground is just as important as rainfall in spring and summer, and the time at which the rain falls in winter is important. Excess of rain in autumn benefits plots 1–A, 3–A and 7–2, and in winter continues to benefit 1–A and 3–A. (3) The curves for 2–O and 2–A are essentially different in character from those of the other plots, and point to the important effect of excess of winter rain in reducing the yield of the plots having phosphate but no alkali salts (*i.e.* no potash, soda, magnesia). (4) Excess of rain at time of sowing is harmful in all cases. (5) The curves of the "O" series (without nitrogen) are much flatter than those of the "A" series, which have a nitrogenous dressing in addition. (6) The indication of summer benefit is not inconsistent with the conclusions of Hooker that a cool summer is desirable for barley. (7) The farmyard manure plot 7–2 bears certain resemblances to the curve for the corresponding plot 2 *b* on Broadbalk.

VII. DISCUSSION.

No attempt has so far been made in this paper to discuss any of the rainfall effects that have been observed, in relation to their possible causes, or to offer suggestions and explanations. We may in conclusion, however, refer briefly to the more important of the effects, adducing such additional information as may be relevant. One of the most interesting features in the whole series of curves is the winter loss due to excessive rain on plot 7–2, and a smaller extent on 2–O and 2–A, *i.e.* those plots which have applied phosphate, with or without a nitrogenous dressing, but no alkali salts. The rainfall curves on these plots are quite

different in character from the others over the winter period. The importance of the phosphatic dressing to the crop has long been recognised. The following table is taken from the earlier study(1).

		Mean yield per acre (1852–70)
Plot 1–A	Single ammonium salts	32·25 ± 1·93 bush.
„ 2–A	Single ammonium salts and superphosphate ...	47·17 ± 1·81 „

It would now appear, however, that this response is not constant, but that it is very materially influenced by the character of the preceding winter. 2–A shows a pronounced loss in yield due to excessive rain over the period December–February, while 1–A shows no such loss. This can only mean that the difference in yield is very much less where the rainfall of the previous winter has been high. Earlier unpublished calculations of R. A. Fisher on the eight adjacent plots of the "AA" and "AAS" series, *i.e* those receiving nitrate of soda (see Table I), had shown that the response to phosphate, while very variable from year to year, was not appreciably influenced by rainfall in the growing season (March–August), but showed a negative correlation with the rainfall of the six months, September–February, prior to sowing. It would be natural to ascribe this effect of rainfall before the seed is in the ground to a depletion of the available nitrogen in the soil, and this may in fact contribute appreciably to the effect observed, but that it does not afford a complete explanation is suggested by two circumstances, (1) that the eight plots used in these calculations all receive a heavy nitrogenous dressing (275 lb. nitrate of soda per acre) with the seed, *i.e.* subsequent to the rain the effect of which is to be explained, and (2) that whereas the effect of winter rainfall on the depletion of nitrogen, which is clearly shown on the wheat plots of Broadbalk(3), there increases to a maximum in mid-winter in correspondence with the observed increases in the proportion of rain that occurs in drainage water, and is inappreciable before October, the effect on barley apparently extends to rainfall over the whole six months.

The differences between plots 1–A and 2–A in our curves may be due to this loss of nitrogen, for this would alter the balance of nitrogen to phosphate to an extent which is more detrimental to the final yield when phosphate is added than when it is omitted. It should be observed in this connection that the curves for 2–O and 2–A resemble that of the farmyard manure plot 7–2 more than any of the others. On the other hand the same effect is less marked on plots 4–O and 4–A which have in addition a dressing of alkali salts (including 200 lb. sulphate of potash per acre).

The other plots are all very much alike except for plots 1–A and 3–A, which stand out as being the only plots that show a benefit from excess of rain during the period November, December and January. These are the plots with a dressing of 200 lb. of ammonium salts, but no phosphate, while 3–A has in addition a dressing of alkali salts. In this latter case the benefit (as also the subsequent spring loss) is more exaggerated. In general the curves seem to point to the winter months being an important time for the crop. Water storage takes place then which may compensate to a large extent for summer drought. The experience of 1929, when the spring and summer were among the driest ever experienced, and yet where the barley crop was everywhere an excellent one, may be cited in this connection.

The next important point to notice from the curves is the influence of excessive rain at the time of sowing and afterwards. The curves nearly all agree in showing that rainfall above the average during the period March–May reduces the final yields. The barley is sown usually in March (average date March 17), but the date has been as early as February 4 (in 1908) and as late as April 27 (1928). There are several causes which may account for this adverse effect:

(1) The deferring of the sowing date owing to bad weather, at the time when the seed should normally go in, or previous to this. This may adversely affect the yield by decreasing the period during which the plant has to grow and ripen, a period which is already a short one. An examination was made of the eighteen years in which the sowing date was on or after March 25. The mean rainfall for March over the seventy-six years 1854–1929 was 1·942 inches. In thirteen of the years examined the rainfall for the month was above the average, ranging from 2·046 inches to 3·747 inches, while in only five of the years was the rainfall under the average. The rainfall was not much under the average in four of these years. In only one year (1926) was a distinctly dry month (0·215 inch) associated with a late sowing date (April 8), and this was due to special soil conditions obtaining at the time.

(2) Excessive rain at time of sowing may cause reduction of plant owing to waterlogging in the soil. It is well known that barley requires to be sown in a fine tilth, and causes (1) and (2) may both operate to the detriment of the final yield if conditions are not good. In fact climatic influences at this early stage in growth are most important in securing speedy germination, and in giving the young plant a good start. Plots 2–O, 2–A and 7–2 also show this adverse effect but not perhaps to the same proportionate extent. These are the plots which reached an

early minimum in February, and from that point on the loss due to excess of rain becomes less and less.

(3) There may be washing out of soluble nitrates. In contrast to the wheat plots of Broadbalk, this does not seem to be an important effect during winter except possibly in the case of plots 2–O, 2–A and 7–2 (see p. 432); but at this period, possibly owing to the fact that the artificial dressings have just been applied, and also because nitrates begin to be needed for the crop in the initial stages of growth, and later, a loss to the final yield due to excessive rain is met with.

The loss due to excess of rain at time of sowing is greater on the plots of the "A" series, *i.e.* those having applied nitrogen, than on those of the "O" series, and this is almost the only feature where the curves resemble those of the wheat plots. This indicates that a rough measure of the amount of loss due to cause (3) might possibly be reached by measuring the difference between corresponding plots of the "O" and "A" series over this important period. The maximum difference between the curves for 3–O and 3–A has already (p. 426) been stated to be 0·55 bushel.

A critical stage in the growth of the plant is during the first six or eight weeks, *i.e.* up to the middle of May. The plant is rapidly expanding, and will need water at this time. On the other hand, much rain may act adversely by leaching out nitrates: these two opposite effects seem to balance one another, and we find a steady reduction in loss due to excessive rain through May and June. A further stage, lasting from the end of May up to the beginning of July, sees the most rapid period of growth in the whole history of the plant, *i.e.* the production of stems and the growth of the ear. Again for this water is required, and we find that about this period the curves all tend to rise above the zero line, *i.e.* excess of rain is if anything slightly beneficial, the benefit being most marked on the farmyard manure plot 7–2 and on the plots dressed with nitrogen. During July and August ripening takes place, and, at any rate over the first half of this period, excess of rain continues to be beneficial. It may be that at this time rain helps on the process of translocation of carbohydrates from the straw to the ear. The average rainfall for July at Rothamsted over the seventy-six harvest years, 1854–1929, is 2·60 inches, and for August 2·64, and the whole of this rain is evidently needed.

All the curves tend to turn downwards during August, due principally to the fact that harvest takes place then (average date August 17) and much rain will occur in this month which is too late to affect the crop. The biggest and earliest effect occurs with the farmyard manure plot:

this may in part be due to lodging. One further interesting effect concerns the plots having a potassic dressing. In the earlier study(1), of the average yields, the conclusion was reached on comparing plots 2 and 5, *i.e.* those having phosphate only and phosphate with a dressing of sulphate of potash, that there was apparently an adverse effect of potash on the yield. The plots compared are, of course, at opposite ends of the field, which is a little unfortunate from the point of view of this comparison, but the conclusion was, that taking this into account the apparent adverse effect persisted. Our rainfall curves show an important difference between plots 2–O and 5–O over the winter period. There is a loss due to excess of rain on 2–O but no such loss with 5–O until after the middle of December. As the only manurial difference is the addition of sulphate of potash, it would appear that this fertiliser has acted in the opposite direction to superphosphate and has tended to counteract the adverse influence of excessive rain on the phosphate plots. If we compare plots 3 and 4 with the corresponding 1 and 2, both on the "O" and the "A" series, the same effect is noticeable, and we have now a better comparison, as the plots compared are adjacent. The differences are more striking in the case of the "A" series, but all are in the same direction, and show that excess of rain is less harmful over the winter period (more beneficial in the case of 1–A and 3–A) where mineral salts, containing potash, have been added.

Acknowledgment is gratefully made to R. A. Fisher for permission to quote certain unpublished calculations on the response of barley to phosphate in relation to winter rainfall, and for helpful advice and criticism during the preparation of this paper; also to F. G. Gregory and L. R. Bishop for help in the discussion of the results.

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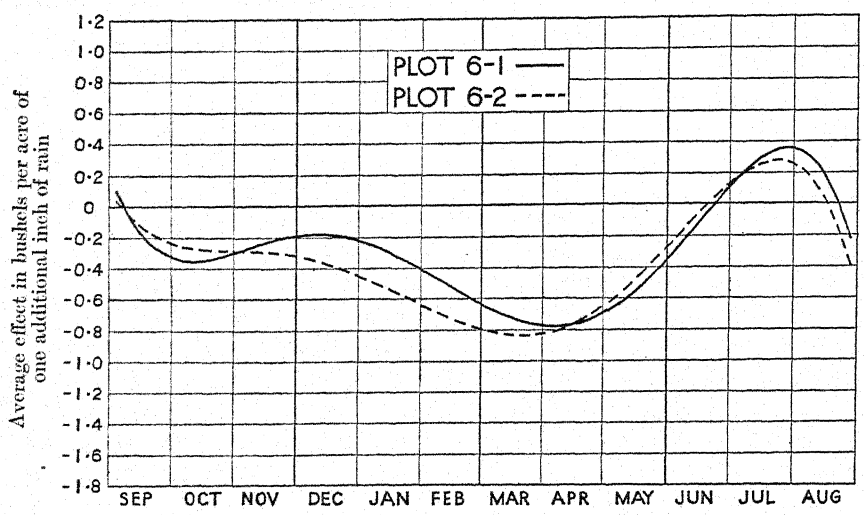

PLOT 6-1
PLOT 6-2
Average effect in bushels per acre of one additional inch of rain
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
-1·6
-1·8
SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG

Fig. 1.

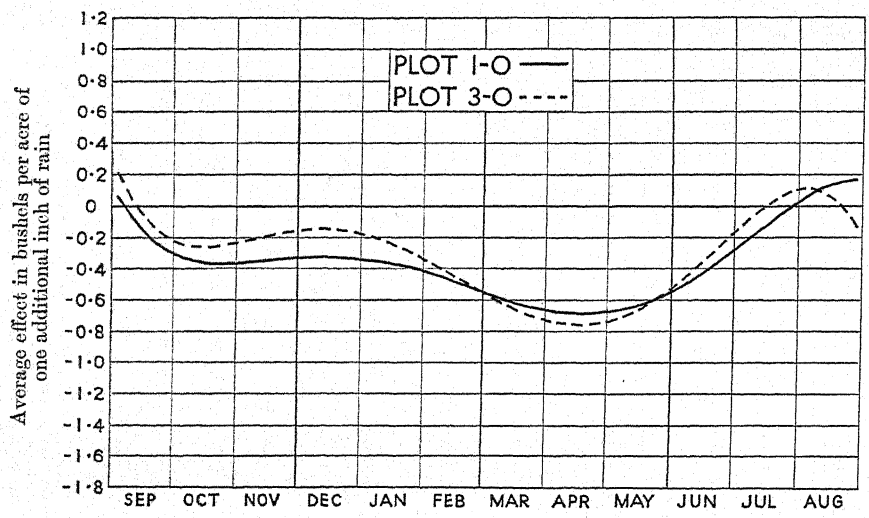

PLOT 1-O
PLOT 3-O
Average effect in bushels per acre of one additional inch of rain
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
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-1·8
SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG

Fig. 2.

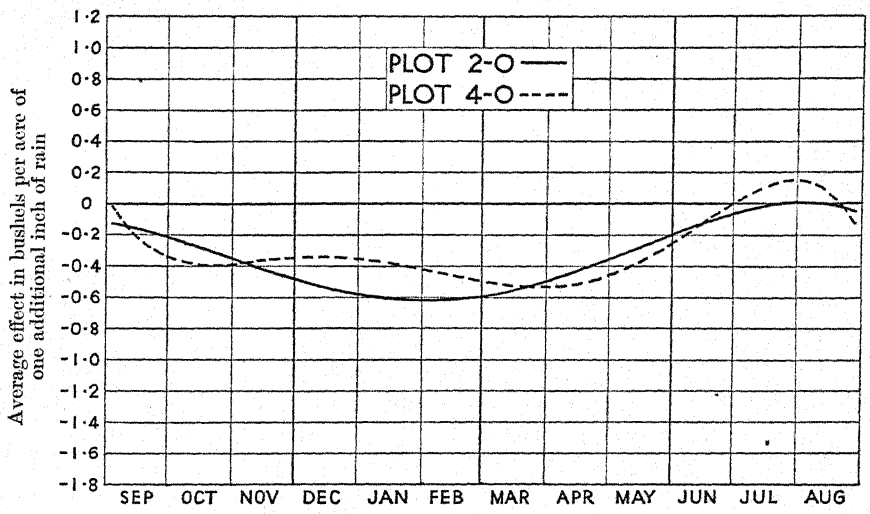

PLOT 2-O
PLOT 4-O
Average effect in bushels per acre of one additional inch of rain
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
-1·6
-1·8
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG

Fig. 3.

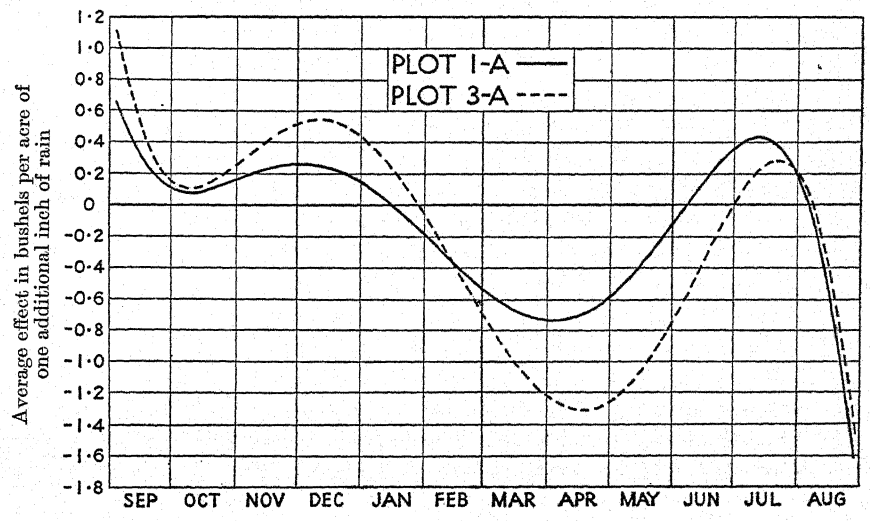

PLOT 1-A
PLOT 3-A
Average effect in bushels per acre of one additional inch of rain
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
-1·6
-1·8
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG

Fig. 4.

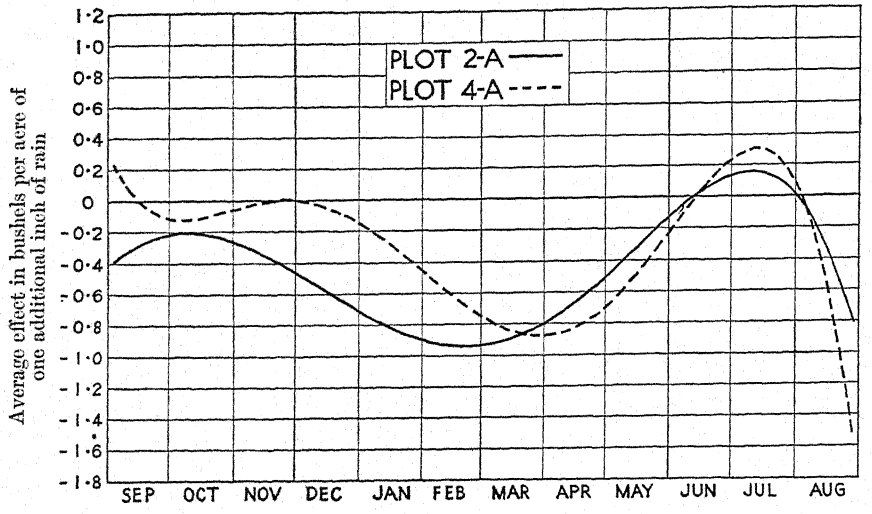

PLOT 2-A
PLOT 4-A
Average effect in bushels per acre of one additional inch of rain
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
-1·6
-1·8
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG

Fig. 5.

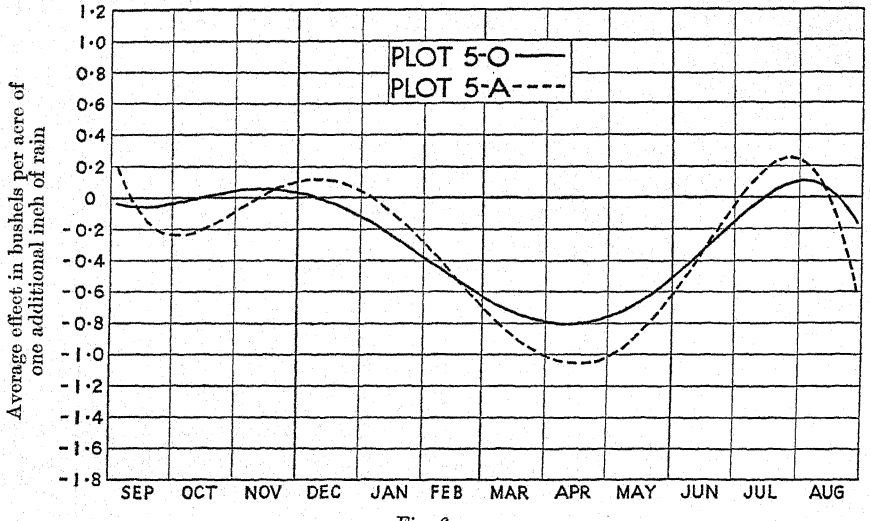

PLOT 5-O
PLOT 5-A
Average effect in bushels per acre of one additional inch of rain
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
-1·6
-1·8
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG

Fig. 6.

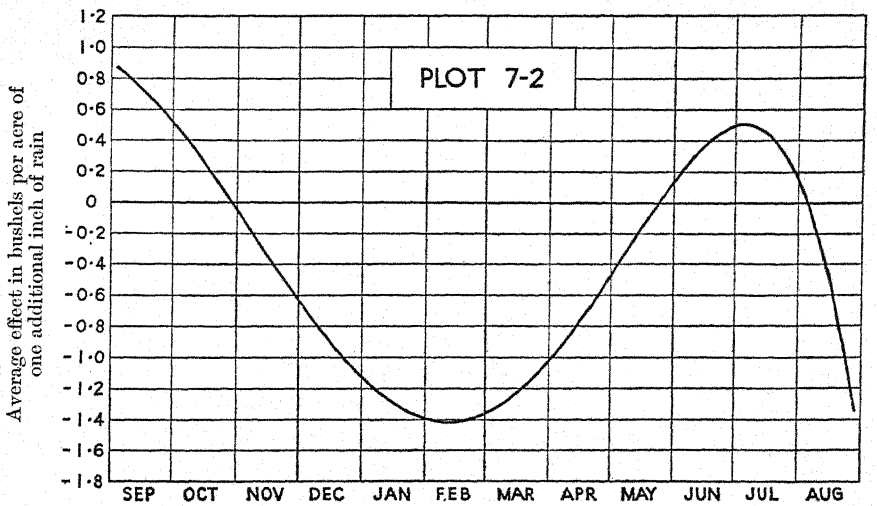


Fig. 7.

(*Received April 15th*, 1930.)

STUDIES IN CROP VARIATION.

VIII. AN APPLICATION OF THE RESISTANCE FORMULA TO POTATO DATA.

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I. Introduction.

A mathematical expression called the Resistance Formula which formulates a yield-factor relationship as suggested by Maskell has been critically tested by Bhai Balmukand (1). The formula postulates that the reciprocal of the yield is the sum of portions, each a function of one nutrient, such as $F(N)$, $F'(K)$, etc. Further, the function of a particular nutrient is inversely proportional to that nutrient in an available form. Based on this hypothesis, the equation for the yield y can be represented thus:

$$\frac{1}{y} = F(N) + F'(K) + F''(P) + \ldots, \text{ etc.},$$

where $$F(N) = \frac{a_n}{n+N}, \quad F'(K) = \frac{a_k}{k+K},$$

a_n, a_k, etc. being the constants varying with the nutrient and the crop; n and k represent the available nitrogen and potash in the unmanured soil, and N and K the quantities added.

II. Material and method of investigation.

The object of this paper is to make a further test of the validity of the Resistance Formula, and use is made of the Rothamsted Potato Experiment on Long Hoos Field, Section I, in 1929. This experiment was designed to give information as to the effect on yield of applying nitrogenous, potassic and phosphatic fertilisers in various quantities. There was a basal dressing of dung at the rate of 14 tons per acre and further nitrogen was supplied as sulphate of ammonia at rates of 0, 0·3 and 0·6 cwt. of nitrogen and potash in the forms of sulphate, muriate and potash manure salts at rates equivalent to 0, 0·5 and 1 cwt. of K_2O per acre. Superphosphate was applied at the rate of 0·4 cwt. of P_2O_5 per acre. The experiment consisted of 81 plots arranged in nine blocks each having plots treated in all combinations of nitrogenous and potassic manures in none, single and double dressings. Further, every

block was equalised with respect to the different kinds of potassic fertilisers, *i.e.* the three plots having single potash had it in the forms of sulphate, muriate and potash salts respectively, and likewise the doubly dressed plots, and each set of these blocks, taken by row or by column, had complete replication both quantitatively and qualitatively for the potash comparison. As for the application of phosphate, each plot was divided into two sub-plots, only one of which, chosen at random, received superphosphate. The area of each sub-plot was 1/90 acre. Analysis showed that the response to potash dressings was the same for all the different qualities, and their yields have, therefore, for present purposes been averaged, yielding Table I below.

Table I. *Potatoes (Ally) Long Hoos, Section I*, 1929.

Average yield in tons per acre (y).

Cwt. of K_2O per acre	Without phosphate (cwt. of nitrogen per acre)			With phosphate (cwt. of nitrogen per acre)		
	0	0·3	0·6	0	0·3	0·6
0	4·52	5·10	5·38	4·79	5·52	5·94
0·5	4·86	5·26	5·41	5·01	5·90	6·28
1·0	4·62	5·30	5·59	4·89	5·77	6·51
Mean		5·12			5·62	
Reciprocal		0·19531			0·17793	

$\frac{1}{2}$ (difference) = 0·00869

Now, the measurement of agreement of the expected yields with those observed affords us a means of judging the accuracy of any hypothesis to be tested. It is therefore necessary to obtain values of the expected yields obeying the Resistance Formula and approximating to the observed.

Crude values of the reciprocals of the expected yields based on the Resistance Formula were first arrived at by averaging the yields obtained for each combination of N and K with and without phosphate, finding their reciprocals and adding to and subtracting from them half the difference of the reciprocals of the means of yields obtained with and without phosphate. This was done by means of the following table.

Table II.

Cwt. of K_2O per acre	(A) Average of the yield with and without phosphate (cwt. of nitrogen per acre)			(B) Reciprocals of (A)		
	0	0·3	0·6	0	0·3	0·6
0	4·66	5·31	5·66	0·21459	0·18832	0·17668
0·5	4·94	5·58	5·84	0·20243	0·17921	0·17123
1·0	4·76	5·54	6·05	0·21008	0·18010	0·16529

Table III. *Reciprocals of the expected yield* 1/m *built up by adding to and subtracting* 0·00869 *from the reciprocals in Table II (B).*

	Without phosphate			Mean (*a*)	With phosphate			Mean (*a*)	
	0·22328	0·19701	0·18537	0·20189	0·20590	0·17963	0·16799	0·18451	
	0·21112	0·18790	0·17992	0·19298	0·19374	0·17052	0·16253	0·17560	
	0·21877	0·18879	0·17398	0·19385	0·20139	0·17141	0·15660	0·17647	
Mean (*b*)	0·21772	0·19123	0·17976	0·19624 (*c*)	(*b*) 0·20034	0·17385	0·16237	0·17886	Mean (*c*)

Table IV (A). *Reciprocals of the expected yields built up from the margins of Table III by use of summation formula* a + b − c.

Without phosphate			With phosphate		
0·22337	0·19688	0·18541	0·20599	0·17950	0·16802
0·21446	0·18797	0·17650	0·19708	0·17059	0·15911
0·21535	0·18884	0·17735	0·19795	0·17146	0·15998

Table IV (B). *Values of first approximation of* m, i.e. *reciprocals of Table IV (A).*

Without phosphate			With phosphate		
4·477	5·079	5·393	4·854	5·571	5·951
4·662	5·320	5·666	5·074	5·862	6·285
4·643	5·296	5·638	5·052	5·832	6·251

For the purpose of improving these expectations we shall further need a table of m^4.

Table IV (C). *Values of* m^4.

	Without phosphate			Total	With phosphate			Total
	402	666	846	1914	551	963	1254	2768
	472	801	1030	2303	663	1181	1560	3404
	465	787	1010	2262	651	1157	1526	3334
Total	1339	2254	2886	6479	1865	3301	4340	9506

			Total
			4682
			5707
			5596
3204	5555	7226	15985

These fourth powers of the crude expectations are then used as weights in obtaining the improved fit, for they are inversely proportional to the variances of the reciprocals in the field trial.

Table V. *Reciprocals of the observed yields* (1/y) *from Table I.*

Without phosphate			With phosphate		
0·22124	0·19608	0·18587	0·20877	0·18116	0·16835
0·20576	0·19011	0·18484	0·19960	0·16949	0·15924
0·21645	0·18868	0·17889	0·20450	0·17331	0·15361

The margins in the following tables are built up by taking the weighted averages of the reciprocals of the observed yields, for rows and columns separately (with and without phosphate) and for the combined tables.

Table VI (A). *Weighted averages of the reciprocals of the observed yields.*

Without phosphate			(a)	With phosphate			(a)
			0·19685				0·18085
			0·19096				0·17066
			0·19002				0·17038
(b) 0·21412	0·19137	0·18306	0·19237 (c)	(b) 0·20402	0·17423	0·15989	0·17353 (c)

Table VI (B).

			(a)	
			0·18739	
			0·17885	
			0·17832	
(b)	0·20824	0·18119	0·16914	0·18117 (c)

The difference of 0·18117 from 0·19237 represents the correction to be applied to the margins of Table VI (B) to get the margins of Table VII (A) below. Likewise the difference of 0·18117 from 0·17353 represents the correction to be applied to the table to get the margins of Table VII (B). The individual nine values of each set are then obtained from the margins by the relation $a + b - c$.

Table VII.

(A) Without phosphate				(B) With phosphate			
			(a)				(a)
0·22566	0·19861	0·18658	0·19859	0·20682	0·17977	0·16772	0·17975
0·21712	0·19007	0·17802	0·19005	0·19828	0·17123	0·15918	0·17121
0·21659	0·18954	0·17749	0·18952	0·19775	0·17070	0·15865	0·17068
(b) 0·21944	0·19239	0·18034	0·19237 (c)	(b) 0·20060	0·17355	0·16150	0·17353 (c)

The margins of Table VI (B) are further improved by applying various corrections which are found by taking the weighted average difference between the reciprocals of the observed and expected values of Tables V and VII respectively. The arithmetic is much facilitated by preparing the following table.

Table VIII. *Differences between the reciprocals of the observed and expected values.*

Without phosphate			With phosphate		
−0·00442	−0·00253	−0·00069	+0·00295	+0·00139	+0·00063
−0·01136	+0·00004	+0·00682	+0·00132	−0·00174	+0·00006
−0·00014	−0·00084	+0·00140	+0·00675	+0·00261	−0·00504

Table IX. *Product of differences and corresponding values of* m^4 *as obtained in Table IV (C).*

	Without phosphate			Total
	−1·77684	−1·68498	−0·58374	−4·04556
	−5·36192	+0·03204	+7·02464	+1·69472
	−0·06510	−0·66108	+1·41400	+0·68782
Total	−7·20386	−2·31402	+7·85486	−1·66302
	With phosphate			Total
	+1·62545	+1·33857	+0·79002	+3·75404
	+0·87516	−2·05494	+0·09360	−1·08618
	+4·39425	+3·01977	−7·69104	−0·27702
Total	+6·89486	+2·30340	−6·80742	+2·39084

Table X. *Corrections obtained by adding margins of Table IX and dividing by marginal* m^4 *in Table IV (C), with and without phosphate combined.*

			−0·00006 +0·00011 +0·00007
−0·00010	0	0·00014	−0·00026 for plots without phosphate +0·00025 for plots with phosphate

The value − 0·00026 is the quotient of − 1·66302 and 6479, while + 0·00025 is the quotient of + 2·39084 and 9506.

The improved values for the reciprocals of the expected yields after applying the corrections are shown in Table XI (A), *e.g.* 0·22524 (without phosphate) and 0·20691 (with phosphate) are obtained by adding − 0·00006, − 0·00010 and − 0·00026 to 0·22566 (without phosphate), and − 0·00006, − 0·00010 and + 0·00025 to 0·20682 (with phosphate) in Tables VII (A) and (B) respectively.

Table XI (A). *Reciprocals of the expected yields* (1/m).

Without phosphate			With phosphate		
0·22524	0·19829	0·18638	0·20691	0·17996	0·16805
0·21687	0·18992	0·17801	0·19854	0·17159	0·15968
0·21630	0·18955	0·17744	0·19797	0·17102	0·15911

Table XI (B). *Values of* m. *Reciprocals of values in Table XI (A).*

Without phosphate			With phosphate		
4·440	5·043	5·365	4·833	5·557	5·951
4·611	5·265	5·618	5·037	5·828	6·262
4·623	5·275	5·733	5·054	5·847	6·285

$\overset{18}{\underset{1}{S}}(y - m)^2 = 0{\cdot}22927$ (from Tables I and XI (B)).

At this stage, two tests were applied to see how far the values of m as obtained in Table XI (B) supply a sufficient fit to the observed yields. If the process is sufficiently complete (i) $S(y-m)^2$ should be approximately at its minimum value; similarly (ii) $Sm^2(y-m)$, taken over all plots treated alike in respect to any one manure, must be small compared to the individual values of $m^2(y-m)$.

Table XI (C). $m^2(y-m)$ *from Tables I and XI (B).*

	Without phosphate			Total	With phosphate			Total
	+1·577	+1·449	+ 0·432	+3·458	−1·004	−0·842	−0·389	−2·235
	+5·294	−0·138	− 6·565	−1·409	−0·685	+2·445	+0·706	+2·466
	−0·064	+0·695	− 4·699	−4·068	−3·989	−2·632	+8·888	+2·267
Total	+6·807	+2·006	−10·832	−2·019	−5·678	−1·029	+9·205	+2·498

Sum of margins			
			+1·223
			+1·057
			−1·801
+1·129	+0·977	−1·627	+0·479

The marginal totals are fairly high when compared with the individual entries, and thus indicate that the values of m as obtained above are not yet sufficiently fitted to the observed yields. Consequently an improved fit of the values of m is now sought by the "Method of Least Squares."

It is required to minimise the quantity $Q = S(y-m)^2$, where y stands for an observed yield and m may be expressed in terms of certain adjustable parameters which may be specified as follows:

$$1/m = N_p + K_q + P_r + C,$$

where P_r takes the two values P and O according as the plot does not or does receive phosphate;

K_q takes three values K, K' and O for none, single and double potash;

N_p takes three values N, N' and O for none, single and double nitrogen.

Then m is obtained from the six arbitrary parameters C, P, K, K', N, N'. The equations to be satisfied on minimising are

$$\frac{dQ}{dC} = \frac{dQ}{dP} = \frac{dQ}{dK} = \frac{dQ}{dK'} = \frac{dQ}{dN} = \frac{dQ}{dN'} = 0.$$

These are non-linear equations in six unknowns but, commencing with the approximate solution already obtained, the solution may be obtained by successive approximations.

Let $C_0, P_0, K_0, K_0', N_0, N_0'$ be any set of approximate values and

suppose $C = C_0 + c$, $P = P_0 + p$, etc., where c, p, k, k', n and n' are the corrections required. Then

$$\text{(i)}\quad 0 = \frac{dQ}{dC} = \frac{dQ}{dC_0} + c\frac{d^2Q}{dC_0^2} + p\frac{d^2Q}{dC_0 dP_0} + k\frac{d^2Q}{dC_0 dK_0} + k'\frac{d^2Q}{dC_0 dK_0'} + n\frac{d^2Q}{dC_0 dN_0} + n'\frac{d^2Q}{dC_0 dN_0'};$$

$$\text{(ii)}\quad 0 = \frac{dQ}{dP} = \frac{dQ}{dP_0} + p\frac{d^2Q}{dP_0^2} + c\frac{d^2Q}{dC_0 dP_0} + k\frac{d^2Q}{dP_0 dK_0} + k'\frac{d^2Q}{dP_0 dK_0'} + n\frac{d^2Q}{dP_0 dN_0} + n'\frac{d^2Q}{dP_0 dN_0'};$$

$$\text{(iii)}\quad 0 = \frac{dQ}{dK} = \frac{dQ}{dK_0} + k\frac{d^2Q}{dK_0^2} + k'\frac{d^2Q}{dK_0 dK_0'} + p\frac{d^2Q}{dK_0 dP_0} + c\frac{d^2Q}{dK_0 dC_0} + n\frac{d^2Q}{dK_0 dN_0} + n'\frac{d^2Q}{dK_0 dN_0'};$$

$$\text{(iv)}\quad 0 = \frac{dQ}{dK'} = \frac{dQ}{dK_0'} + k'\frac{d^2Q}{dK_0'^2} + k\frac{d^2Q}{dK_0 dK_0'} + p\frac{d^2Q}{dK_0' dP_0} + c\frac{d^2Q}{dK_0' dC_0} + n\frac{d^2Q}{dK_0' dN_0} + n'\frac{d^2Q}{dK_0' dN_0'};$$

$$\text{(v)}\quad 0 = \frac{dQ}{dN} = \frac{dQ}{dN_0} + n\frac{d^2Q}{dN_0^2} + n'\frac{d^2Q}{dN_0 dN_0'} + p\frac{d^2Q}{dN_0 dP_0} + c\frac{d^2Q}{dN_0 dC_0} + k\frac{d^2Q}{dN_0 dK_0} + k'\frac{d^2Q}{dN_0 dK_0'};$$

$$\text{(vi)}\quad 0 = \frac{dQ}{dN'} = \frac{dQ}{dN_0'} + n'\frac{d^2Q}{dN_0'^2} + n\frac{d^2Q}{dN_0 dN_0'} + p\frac{d^2Q}{dN_0' dP_0} + c\frac{d^2Q}{dN_0' dC_0} + k\frac{d^2Q}{dN_0' dK_0} + k'\frac{d^2Q}{dN_0' dK_0'}.$$

Thus we have six equations which are linear in c, p, k, k', n and n' with known coefficients, for

$\frac{dQ}{dC_0} = -2S(y-m)\frac{dm}{dC_0} = 2\overset{18}{\underset{1}{S}}\{m^3(y-m)\}$, the summation being over all the eighteen plots;

$\frac{dQ}{dP_0} = 2\overset{9}{\underset{1}{S}}m^2(y-m)$ over plots without phosphate;

$\frac{dQ}{dK_0} = 2\overset{6}{\underset{1}{S}}m^2(y-m)$ over plots without potash;

$\frac{dQ}{dK_0'} = 2\overset{6}{\underset{1}{S}}m^2(y-m)$ over single potash plots;

$\frac{dQ}{dN_0} = 2\overset{6}{\underset{1}{S}}m^2(y-m)$ over plots without nitrogen;

$\frac{dQ}{dN_0'} = 2\overset{6}{\underset{1}{S}}m^3(y-m)$ over single nitrogen plots.

These quantities can all be obtained readily from Table XI (C).

$\frac{d^2Q}{dC_0^2} = 2\frac{d}{dC_0}\overset{18}{\underset{1}{S}}m^2(y-m) = 2\overset{18}{\underset{1}{S}}\{m^4 - 2m^3(y-m)\}$ summation being over all the eighteen plots;

$\frac{d^2Q}{dC_0 dP_0} = 2\overset{9}{\underset{1}{S}}\{m^4 - 2m^3(y-m)\}$ over plots without phosphate;

$\frac{d^2Q}{dP_0 dK_0'} = 2\overset{3}{\underset{1}{S}}\{m^4 - 2m^3(y-m)\}$ over plots without phosphate but with single dressing of potash;

$\frac{d^2Q}{dC_0 dK_0} = 2\overset{6}{\underset{1}{S}}\{m^4 - 2m^3(y-m)\}$ over plots without potash;

and so on

$$\frac{d^2Q}{dK_0 dK_0'} = \frac{d^2Q}{dN_0 dN_0'} = 0.$$

The values of $\frac{d^2Q}{dC_0^2}$, $\frac{d^2Q}{dC_0 dP_0}$, etc., are obtained from the following table:

Table XII. $m^4 - 2m^3(y-m)$.

Without phosphate			Total	With phosphate			Total
374·637	632·166	823·828	1830·631	555·304	962·938	1258·787	2777·029
403·209	769·857	1069·923	2242·989	650·589	1125·184	1528·819	3304·592
457·355	766·947	1134·130	2358·432	692·766	1199·534	1448·610	3340·910
Total 1235·201	2168·970	3027·881	6432·052	1898·659	3287·656	4236·216	9422·531

Sum of margins

			4607·660
			5547·581
			5699·342
3133·860	5456·626	7264·097	15854·583

The values of $\frac{dQ}{dC_0}$, $\frac{d^2Q}{dC_0^2}$, $\frac{d^2Q}{dC_0 dP_0}$, ..., etc. when substituted in the six equations enable them to be solved for c, p, k, k', n and n', with the following results:

$c = -0{\cdot}000258$; $k' = +0{\cdot}000028$;

$p = +0{\cdot}000158$; $n = -0{\cdot}000160$;

$k = -0{\cdot}000045$; $n' = +0{\cdot}000022$.

The table of corrections is then built up from the relation

$$c + x + y + z,$$

where x takes the values p and o according as it is applied to plots without phosphate and plots with phosphate respectively. Similarly y takes the values k, k' and o for none, single and double potash; z takes the values n, n' and o for none, single and double nitrogen.

Table XIII. *Table of corrections.*

Without phosphate (0·000)			With phosphate		
−31	−12	−15	−46	−28	−30
−23	−05	−07	−39	−21	−23
−26	−08	−10	−42	−24	−26

Table XIV (A). *Corrected values of the reciprocals of the expected yields by adding values in Tables XI (A) and XIII.*

Without phosphate			With phosphate		
0·22493	0·19817	0·18623	0·20645	0·17968	0·16775
0·21664	0·18987	0·17794	0·19815	0·17138	0·15945
0·21604	0·18927	0·17734	0·19755	0·17078	0·15885

Table XIV (B). *Values of* m *reciprocals of Table XIV (A).*

Without phosphate			With phosphate		
4·446	5·046	5·370	4·844	5·565	5·961
4·616	5·267	5·620	5·047	5·835	6·271
4·629	5·283	5·639	5·062	5·855	6·295

$$\overset{18}{\underset{1}{S}}(y - m)^2 = 0{\cdot}20899.$$

Table XIV (C). m^2 (y − m) *from Tables I and XIV (B).*

	Without phosphate			Total	With phosphate			Total
	+1·466	+1·371	+0·297	+3·134	−1·262	−1·408	−0·755	−3·425
	+5·200	−0·187	−6·944	−1·931	−0·934	+2·213	+0·332	+1·611
	−0·188	+0·462	−1·555	−1·281	−4·408	−2·931	+8·511	+1·172
Total	+6·478	+1·646	−8·202	−0·078	−6·604	−2·126	+8·088	−0·642

Combined marginal totals

			−0·291
			−0·320
			−0·109
−0·126	−0·480	−0·114	−0·720

The marginal totals are now much reduced, indicating that the value of m has been further improved.

Test of Goodness of Fit.

So far, we have obtained the theoretical values based on the Resistance Formula. It now remains to see whether these values are in agreement with the values obtained by the experiment. The method known as the

analysis of variance developed by Dr R. A. Fisher(2) is applied to test the validity of the hypothesis. From the analysis of variance for this experiment it is found that after apportioning fractions of the total sum of squares of deviations of the yields from their general mean to various known causal factors, the following residual amounts are left for (i) the N and K error, and (ii) the phosphate error. (For an explanation of a similar analysis but with only three blocks see Wishart and Clapham(3)).

Analysis of variance due to:	Degrees of freedom	Sum of squares	Mean square
(i) Non-adjacent sub-plots (N and K error)...	60	231278·18	3854·64
(ii) Adjacent sub-plots (phosphate error) ...	60	59271·89	987·86

These figures are based on the yields in $\frac{1}{4}$ lb. units per 1/90th of an acre. Expressed in tons per acre, the mean squares for non-adjacent and adjacent sub-plots are 0·38800 and 0·09968 respectively. But the yields in our tables are based on the means of nine sub-plots; so to make the figures comparable we have to divide them by nine. Thus

Analysis of variance due to:	Degrees of freedom	Sum of squares	Mean square
(i) Non-adjacent sub-plots (N and K error)...	60	2·5928	0·04321
(ii) Adjacent sub-plots (phosphate error) ...	60	0·6648	0·01108

There are seventeen degrees of freedom for the eighteen values of the table out of which five have been accounted for in fitting the constants, leaving twelve degrees of freedom in which the observed values might differ from expectations. The twelve degrees of freedom can further be split up into (i) eight for differences from expectation due to fertility of adjacent sub-plots, and (ii) four for the non-adjacent ones. The total sum of the squares of the deviations of the expected yields from the observed ones is

$$\overset{18}{\underset{1}{S}}\,(y - m)^2 = 0{\cdot}20899.$$

The sum of the squares of the deviations of the expected yields from the observed values for eight degrees of freedom due to fertility of adjacent sub-plots was calculated from the following tables.

Table XV. (y − m) *from Tables I and XIV (B).*

Without phosphate			With phosphate		
+0·074	+0·054	+0·010	−0·054	−0·045	−0·021
+0·244	−0·007	−0·210	−0·037	+0·065	+0·009
−0·009	+0·017	−0·049	−0·172	−0·085	+0·215

Table XVI. *Differences, phosphate—no phosphate, of* (y − m) *from Table XV.*

−0·128	−0·099	−0·031
−0·281	+0·072	+0·219
−0·163	−0·102	+0·264

½ (sum of squares of the above nine figures) = 0·13270.

The difference of 0·13270 from 0·20899 represents the sum of the squares of the deviations of the expected yields from the observed values with four degrees of freedom for the non-adjacent plots.

If the Resistance Formula has been satisfactorily fitted, the mean sum of squares with the eight degrees of freedom should not be significantly larger than that obtained from the field error for the phosphate comparison; similarly, the mean square of deviations with four degrees of freedom should not be significantly larger than that obtained for the N and K comparison in the field experiment. We have:

Analysis of variance due to:	Degrees of freedom	Sum of squares	Mean square	½ log$_e$ (mean square + 100)
(i) Resistance formula	4	0·0763	0·01907	0·3227
Non-adjacent sub-plots (N and K error)	60	2·5928	0·04321	0·7317

In this case the mean square from the Resistance Formula is actually less than that for the corresponding field error, $z = -0·4090$.

Analysis of variance due to:	Degrees of freedom	Sum of squares	Mean square	½ log$_e$ (mean square + 100)
(ii) Resistance formula	8	0·1327	0·01659	0·2531
Adjacent sub-plots (P error) ...	60	0·6648	0·01108	0·0512

In this case, although the mean square of deviations for adjacent sub-plots is greater than the mean square for the corresponding field error, it is not significant, as is shown by the value of z. To reach the 5 per cent. point the value of z is required to be 0·3702. It is evident that the Resistance Formula has fitted the data satisfactorily in this case.

III. Constants of the Resistance Formula.

Bh. Balmukand has shown in the data examined by him that $F(N)$, $F'(K)$, etc. can be taken to be of the form $\frac{a_n}{n+N}$ and $\frac{a_k}{k+K}$, where a_n and a_k are the constants varying with the nutrient and the crop, while n and k represent the available nitrogen and potash in the unmanured soil. The fitness of this special formula cannot be tested from our data, but the values of the constants n, a_n, k, a_k are calculated by means of this formula and their standard errors determined by the Method of Maximum Likelihood as developed by him. In view of the fact that there are only two levels of phosphate the values for the

constants p and a_p cannot be determined. From the Table XIV (A) we find that the difference between the reciprocals of the expected yields for no nitrogen and 0·3 cwt. of nitrogen is practically 0·02677—any departure from constancy in the last place being really due to the neglect of the sixth decimal place—and that between those of 0·3 and 0·6 of nitrogen is 0·01193. Similarly for potash we have 0·0083 and 0·0006.

We have the following equations:

$$\text{(i)} \qquad \frac{a_n}{n} - \frac{a_n}{n+0{\cdot}3} = 0{\cdot}02677;$$

$$\text{(ii)} \qquad \frac{a_n}{n+0{\cdot}3} - \frac{a_n}{n+0{\cdot}6} = 0{\cdot}01193.$$

Solving, we find

$n = 0{\cdot}4823$ cwt. of $N_2 = 2{\cdot}305$ cwt. of sulphate of ammonia per acre;

$a_n = 0{\cdot}0337$ cwt. of $N_2 = 0{\cdot}161$ cwt. of sulphate of ammonia per ton of potato.

(1 cwt. of nitrogen is contained in 4·78 cwt. of sulphate of ammonia.)

Similarly

$$\text{(i)} \qquad \frac{a_k}{k} - \frac{a_k}{k+0{\cdot}5} = 0{\cdot}0083;$$

$$\text{(ii)} \qquad \frac{a_k}{k+0{\cdot}5} - \frac{a_k}{k+1} = 0{\cdot}0006.$$

On solving, we have

$k = 0{\cdot}0779$ cwt. of $K_2O = 0{\cdot}157$ cwt. of sulphate of potash per acre;

$a_k = 0{\cdot}0007$ cwt. of $K_2O = 0{\cdot}0015$ cwt. of sulphate of potash per ton of potato.

(1 cwt. of K_2O is contained in 2·02 cwt. of sulphate of potash.)

Standard errors of the constants.

The calculation of the standard errors was as follows:

Table XVII (A). *Values of* m^4 *as obtained from Table XV (B).*

Without phosphate			Total	With phosphate			Total
390·671	648·405	831·382	1870·458	550·480	959·401	1262·843	2772·724
453·988	769·452	997·480	2220·920	648·670	1159·205	1540·456	3348·331
461·559	779·242	1011·056	2251·857	656·584	1175·585	1570·553	3402·722
Total 1306·218	2197·099	2839·918	6343·235	1855·734	3294·191	4373·852	9523·777

Combined marginal totals

				w_{kt}
				4643·182
				5569·251
				5654·579
w_{nt}	3161·952	5491·290	7213·770	15867·012

Table XVII (B).

	w_{nt}	$n+N_t$ (cwt. of sulphate of ammonia)	$\frac{w_{nt}}{n+N_t}$	$\frac{w_{nt}}{(n+N_t)^2}$	$\frac{w_{nt}}{(n+N_t)^3}$	$\frac{w_{nt}}{(n+N_t)^4}$
1	3161·952	2·305	1371·78	595·13	258·19	112·01
2	5491·290	3·739	1468·65	392·79	105·05	28·10
3	7213·770	5·173	1394·50	269·57	52·11	10·77
Total	15867·012	—	4234·93	1257·49	415·35	150·88

Table XVII (C).

t	w_{kt}	$k+K_t$ (cwt. of sulphate of potash)	$\frac{w_{kt}}{k+K_t}$	$\frac{w_{kt}}{(k+K_t)^2}$	$\frac{w_{kt}}{(k+K_t)^3}$	$\frac{w_{kt}}{(k+K_t)^4}$
1	4643·182	0·157	29574·41	188372·02	1199821·80	7642177·11
2	5569·251	1·167	4772·28	4089·36	3504·16	3002·71
3	5654·579	2·177	2597·42	1193·12	548·06	251·75
Total	15867·012	—	36944·11	193654·50	1203874·02	7645431·57

The values of the standard errors were obtained by using the formula given by Bh. Balmukand.

$$V(a_n) = \sigma^2 \frac{\begin{vmatrix} \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^4} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^2} \\ \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^2} & \overset{3}{S}_1 w_{nt} \end{vmatrix}}{\begin{vmatrix} \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^2} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^3} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)} \\ \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^3} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^4} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^2} \\ \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^2} & \overset{3}{S}_1 w_{nt} \end{vmatrix}},$$

$$V(n) = \frac{\sigma^2}{(a_n)^2} \begin{vmatrix} \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)^2} & \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)} \\ \overset{3}{S}_1 \frac{w_{nt}}{(n+N_t)} & \overset{3}{S}_1 w_{nt} \end{vmatrix} \div D,$$

where D is the denominator of the previous fraction.

Substituting the respective values:

$\sigma_n = 0{\cdot}1158$ cwt. of sulphate of ammonia,

$\sigma_{a_n} = 0{\cdot}1182$ cwt. of sulphate of ammonia.

Similarly:

$\sigma_{(k)} = 1{\cdot}1552$ cwt. of sulphate of potash,

$\sigma_{a_k} = 0{\cdot}0122$ cwt. of sulphate of potash.

Expressing the constants with their standard errors in lb. of nitrogen and K_2O, we have:

n = 54·02, S.E. 2·71, k = 8·70, S.E. 64·05,

a_n = 3·77, S.E. 2·77, a_k = 0·08, S.E. 0·68.

With the exception of n, for which the standard error is only 5 per cent., the standard errors we have obtained for the constants of the Resistance Formula are very high. The values of the constants together with their standard errors are set out in the following table and the results previously obtained by Bh. Balmukand, converted into pounds of nitrogen and K_2O respectively, are added for comparison.

Table XVIII.

	n	S.E.	a_n	S.E.	k	S.E.	a_k	S.E.
Long Hoos (Ally) 1929, dung (14 tons)	54·02	2·71	3·77	2·77	8·70	64·05	0·08	0·68
Stackyard (Kerr's Pink), 1926 ...	40·71	19·91	2·31	1·44	26·61	33·27	0·49	0·71
Seale Hayne: dung (10 tons)	38·89	9·37	3·96	1·12	62·65	32·16	1·43	0·94
Seale Hayne: undunged, 1927 ...	13·35	5·15	1·85	0·80	−5·55	4·99	0·42	0·35

IV. SUMMARY AND CONCLUSION.

The table of results given in the last section shows that for all experiments for which the Resistance Formula has been fitted the values of the constants are consistent, bearing in mind the standard errors to which these values are subject. The constants for nitrogen may be held to have been determined with some approach to precision, but those for potash are not so well determined. We find at Rothamsted, for example, that the crop responds to a moderate dressing of potash, but higher dressings do not usually improve the yields any further. This fact limits the precision of the formula. The reader is asked to refer at this point to the full descriptions as to the meaning to be attached to the constants of the formula given by Bh. Balmukand in the earlier paper (1). The constants a_n and a_k, called the importance factors, are interpreted as determining the capacity of the crop to recover the particular nutrient out of the soil. The chemical analysis of the tubers grown under nitrogen and potash starvation conditions should, therefore, furnish us with values which should be of the same order of magnitude as obtained by the formula. The minimum nitrogen percentage figure is 0·204, which when reduced to our units is equivalent to 4·57 lb. of nitrogen per ton of potato. The values obtained from the Resistance Formula are as close as the standard errors allow us to expect, and are also of the same order of magnitude as the chemical composition of the tubers demands.

The 1929 potato experiment studied in this paper may be regarded

as confirming the conclusions of Balmukand's paper as to the possibility of fitting the Resistance Formula to experimental data. It has confirmed the values previously reached for the nitrogen constants on a dunged soil, but the corresponding constants for potash cannot yet be regarded as well determined from any experiment so far examined. It so happens that only a moderate response to potash in the case of potatoes occurs on the Rothamsted soil, and this has led in the past few years to a reduction in the quantity applied in order to have a number of suitable levels. This policy has not yet succeeded, and one may anticipate that the unit dressing will be still further reduced in future. The precision of the Resistance Formula depends upon a suitable number of such levels being incorporated into any experiment, at each of which some response is shown to the dressing. So we can only hope to improve the precision of the determination of the potash constants by still further reducing the unit dressing. It is hoped that an experiment on these lines now being initiated at Rothamsted will furnish the information sought.

Finally it is a pleasure to record my indebtedness to Dr R. A. Fisher, who suggested the problem of this paper and whose help at various stages has been invaluable, and also to Dr J. Wishart of the Rothamsted Experimental Station for much valuable advice and criticism.

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(*Received April 15th*, 1930.)

STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

I. INTRODUCTORY

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The organic matter of the soil has engaged the attention of soil investigators ever since the latter part of the eighteenth century. The volume of literature on the subject is enormous, and perhaps in a greater degree than in any other branch of soil work we find here examples of the tendency to draw definite conclusions from inconclusive evidence. This is not surprising when it is remembered that perhaps the most important part of the soil organic matter, the humic matter, is a high molecular organic colloid, the investigation of which takes us at once into the most difficult fields of organic and of physical chemistry—fields in which the worker often needs to use special methods of experimentation which have become available only during recent years. Moreover, the study of humic matter is rendered particularly difficult, since it is a *secondary* product derived from the complex organic constituents of plant residues, themselves of unknown or little-known constitution, and since it is not known from which of these plant constituents it is derived.

The literature of the soil organic matter has been fully reviewed by several workers in recent years, and no attempt will be made to discuss that literature exhaustively here.

The publication by Odén in 1919 of a series of papers on humic matter(1) marked a definite epoch in the study of the soil organic matter. The reader seeking a critical account of earlier work on the subject is referred to the historical survey and bibliography given by Odén, and to the later publication of Waksman(2), who has made outstanding contributions to the subject during the last few years.

The organic matter of the soil may be classified into two parts, the humic matter and the non-humic matter. The usage of the terms "humus" and "humic" is subject to wide variations, so that before going any further it is necessary to explain what is meant by the above

[1] The investigations dealt with in this series of papers were carried out by or under the direction of the author up to the time of his leaving the Rothamsted Experimental Station in 1927.

classification. In this series of papers it is proposed to avoid altogether the use of the term "humus", owing to the widely varying meanings attached to this word by different workers; the term "humic matter" will be used as a description of the *dark-coloured high-molecular colloidal organic matter* which is a characteristic constituent of the soil; *non-humic matter* in the soil includes not only the colourless organic substances, largely soluble, which result from the chemical and biological decomposition of plant and animal residues and from the further decomposition of humic matter itself, but also the undecomposed parts of those residues containing cellulose, lignin, waxes, and so on, which are also colourless. This division is easier to make on paper than it is in the laboratory; present methods are not sufficiently advanced to render it capable of complete attainment. However, as a working division, it is tolerably satisfactory.

A part of the humic matter, when freed from associated metals (such as calcium), is readily soluble in cold, weak alkali, and is re-precipitated from that solution by acids. This part of the humic matter can then be further divided into a part soluble in alcohol and an insoluble part. The term "humic acid" will be used in this series of papers for the part insoluble in alcohol. Odén used the name of "hymatomelanic acid", originally used by Hoppe Seyler, for the alcohol-soluble fraction, but this name is avoided in these papers, not only on account of its awkwardness, but because it is not considered that the fraction to which it refers is sufficiently well-defined.

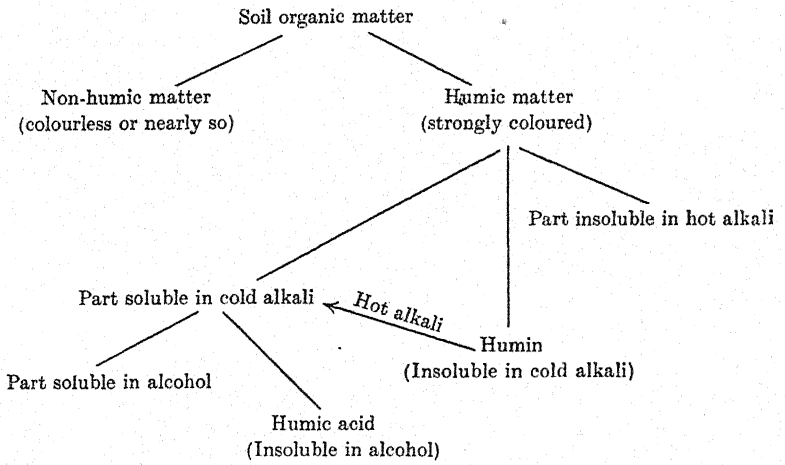
Of that part of the humic matter not dissolved by cold, weak alkali, a portion is soluble in hot alkali. When it is convenient to use a name for this fraction, it will be designated as "humin", in the same sense as that used by Odén. Acidification of the solution obtained by treatment of humin with hot alkali causes the separation of the dissolved humic matter in a form, of which the alcohol-insoluble part has not up to now been shown to be different from the humic acid obtained from the cold alkaline extract of the same soil, and which will be included under the name of "humic acid", without prejudice to the possibility of its being shown subsequently to differ from that derived from cold alkali extraction.

The solution remaining after acidification of an alkaline extract still contains some weakly coloured organic substances, to which Odén gave the name of "fulvic acids". These weakly coloured bodies have not been studied in these investigations, and for the purposes of these papers they are not regarded as humic matter (although they possibly represent early

stages in the degradation of humic matter), but as a part of the non-humic matter.

After extraction of the soil with hot alkali a portion of the organic matter remains undissolved. Nothing is known regarding this insoluble part, and until it has been characterised it is unnecessary and indeed misleading to give it a definite name.

The nomenclature adopted, and the relation of the various fractions to each other, are shown in the following diagram.



Special mention must be made of the well-known work of Schreiner and his co-workers at the Bureau of Soils of the United States Department of Agriculture(3). This brilliant series of investigations was concerned almost wholly with the non-humic matter of the soil. So comprehensive was this work that little has been added to it subsequently.

The work described in this series of papers is concerned chiefly with the humic matter and with the carbon and nitrogen economy of the soil. The non-humic matter is considered chiefly only in its bearings on the origin and isolation of the humic matter. The non-humic matter tends to accumulate at the two ends of the extraction process, representing the most soluble and the least soluble parts. A little is directly extracted by water or weak acids, but much more remains dissolved in the colourless or faintly coloured liquid obtained when an alkaline extract of the soil is freed from humic matter by precipitation with acid. The part of the soil organic matter insoluble in alkali usually contains, in addition to insoluble humic matter, some non-humic matter in the form of the more resistant undecomposed constituents of animal and plant residues.

The work of Odén was chiefly concerned with humic acid. The long-standing controversy regarding the acid nature of humic acid was settled by Odén's demonstration that this substance does indeed behave as a true acid, judged by its behaviour in conductometric and potentiometric titrations. This work, taken in conjunction with the colloidal nature of the material, definitely places humic acid in that class of colloidal electrolytes which have been termed "acidoids" by Michaelis—high-molecular acids that dissociate to give a colloidal anion. This is not the place to consider the bearings of these results on the electrokinetic and ionic-exchange phenomena of humic acid—except to point out that humic acid is thus brought into the same category as the inorganic colloidal material of the soil, the clay acids, which are now known to behave as acidoids.

Signal as was this contribution of Odén's, in certain other directions his work has conduced to erroneous impressions. Thus his estimation of the "equivalent weight" of humic acid has tended rather to the idea that humic acid may be regarded as a chemical individual. Odén was careful not to state that view in his published work, but nevertheless such a view is rather implied thereby. Further, the fact that Odén's preparation was almost free from nitrogen has rather diverted attention from the question of the nitrogen content of humic matter.

It cannot be too strongly emphasised that there is, up to now, no satisfactory evidence that any of the preparations that have been made from the humic matter of the soil may be regarded as chemical individuals. Nevertheless, chemical or physical methods enable the humic matter to be fractionated into different groups of substances with certain well-defined physical and chemical characteristics. Although the time when the isolation of well-defined chemical substances from the humic matter will be effected may still be distant, the characterisation of the different groups of substances which are comprised under such names as "humin", "humic acid", etc., is a necessary preliminary to such isolation, and can throw considerable light on the origin, nature and functions of the humic matter of the soil.

The investigations that form the subject of this series of papers were carried out between the years 1920 to 1927 in the chemical department of the Rothamsted Experimental Station, by the author or under his direction. The development of this work may conveniently be summarised briefly here, in order to explain the relation of the different parts of the series to one another.

The first stages of this work, which are described in the earlier papers

of the series, had the primary object of attempting to ascertain the parent substance or substances of the humic matter of the soil. Evidence was obtained in favour of the derivation of humic matter from lignin. When this work had reached a certain stage, it became clear that further progress could not be made without a careful investigation of the organic nitrogen compounds of the soil. This work led in turn to attempts to fractionate "humic acid", and, in particular, to separate it into nitrogenous and non-nitrogenous constituents. A closely related aspect of the subject was the study of the changes in the nitrogen content of the soil, under long-continued manuring with organic or with inorganic manures. Finally, this led to the consideration of the biological factors in the breakdown of plant and animal residues in the soil, in their relation to the humification process. Based on the results recorded in the preceding papers, there is developed in the last paper of the series a hypothesis regarding the mode of formation and the nature of the humic matter—nitrogen complex of the soil, and the influence of that complex, and of its formation and subsequent breakdown, on the carbon and nitrogen economy of the soil and on soil fertility.

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(*Received March 3rd*, 1930).

STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

II. THE EXTRACTION OF THE ORGANIC MATTER OF THE SOIL WITH ALKALI.[1]

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The permanent wheat and root experiments on Broadbalk Field and Barnfield at Rothamsted afford an unrivalled opportunity of studying the influence of widely varying and long-continued manurial and cultural treatment on the organic matter of the soil. As a result of the different treatments which the various plots in the above experiments have received consistently for a large number of years, very marked differences in carbon content and in fertility have arisen. The investigations described in this paper had the object of comparing the properties of the organic matter of the soils of certain of these plots. It was thought that the quantitative differences in carbon content and in fertility of these soils might be accompanied by qualitative differences which would affect the solubility of the soil organic matter in alkali.

Table I. *Particulars of soils used.*

Soil from		Manurial treatments (annual) (per acre)
Barnfield. Permanent roots. Since 1856 (1856–75 swedes. 1876 onwards, mangolds)	Plot 1 C	Dung 14 tons since 1856 + rape cake 2000 lb. since 1861*
	Plot 8 O	No manure since 1856
	Plot 8 A	Ammonium salts, 400 lb. since 1856†
Broadbalk Field. Permanent wheat. Since 1843	Plot 2 B	Dung 14 tons
	Plot 3	No manure (since 1839)
	Plot 7	Ammonium salts 400 lb., superphosphate 392 lb., potassium sulphate 200 lb., sodium and magnesium sulphate each 100 lb.

* From 1856–61 this plot received 3000 lb. sawdust per acre annually instead of rape cake.

† From 1856–61 the rate of application of ammonium salts was only 200 lb. per acre.

[1] For Part I of this series see the preceding paper (I).

[2] One of the authors (C.W.B.A.) was awarded the degree of Doctor of Philosophy by the University of London in 1925, for a Thesis embodying the results described in this paper.

[3] The investigations dealt with in this series of papers were carried out by or under the direction of the senior author (H.J.P.) up to the time of his leaving the Rothamsted Experimental Station in 1927.

The soils selected for examination are shown in Table I, which also summarises the treatments applied to the plots from which the soils were taken. For fuller particulars of the treatment of these plots and the crop yields, see (2).

The samples of soil were taken in the autumn of 1921. The sample from each plot consisted of a mixture of twelve cores from points uniformly distributed over the whole plot.

That the differences in manurial treatment detailed in Table I have brought about considerable differences in the carbon content of the soil is seen from Table II.

Table II. *Carbon content of soils used.*

Soil		Organic carbon % on oven-dry soil: Surface soil	Subsoil
Barnfield:	Plot 1 C	2·85	1·02
	Plot 8 O	0·81	0·54
	Plot 8 A	0·84	—
Broadbalk:	Plot 2 B	2·91	1·04
	Plot 3	0·89	0·62
	Plot 7	1·10	0·64

In the study of these soils the endeavour was to find a method of broadly fractionating the soil organic matter, simple enough to be applied in routine soil analysis. The method adopted was to treat the soil with dilute acid, and then to determine the total carbon content of the acid-treated soil and the carbon content of the extracts obtained with cold and hot alkali under standard conditions.

EXPERIMENTAL.

Before extracting a soil with alkaline solvents, for the estimation of alkali-soluble humic matter, it is usual to treat it with weak hydrochloric acid in order to decompose carbonates and liberate humic matter combined with calcium and other metals. The presence of chlorides is objectionable in a combustion process, owing to the liberation of chlorine, but sulphuric acid, although unobjectionable when a wet combustion method is to be used, was found for other reasons to be unsuitable. Further, the washing of soil on filter paper after treatment with acid is undesirable, owing to the risk of introducing fibres of cellulose into the soil. The method finally adopted consisted of treatment with hydrochloric acid, followed by washing by decantation.

When soil is shaken with an excess of dilute hydrochloric acid, the first stage of the neutralisation, corresponding to the decomposition of

carbonates and "humates," and the bringing into solution of exchangeable bases, is rapid. There follows a slower neutralisation owing to more deep-seated action. It is only the first rapid stage which is needed as a preliminary to the extraction of humic matter. It was found that with the soils dealt with in this investigation, shaking for 2 hours with 2 per cent. hydrochloric acid was sufficient to complete this first stage.

The standard conditions adopted were:

An amount of air-dried fine earth corresponding to 10 gm. of the oven-dried material was shaken for 2 hours with 25 c.c. of 2 per cent. hydrochloric acid, set aside for a short time, and a portion filtered. The amount of acid which had been neutralised was determined approximately by titration. In the subsequent treatments of further portions of this soil, 5 c.c. more than the latter amount of acid was employed, in order that the concentration of residual acid would be about the same in all cases. After 2 hours' shaking the bottles were put aside until the supernatant liquid was clear. This liquid was then removed as completely as possible by aspiration. The bottles were then filled up with freshly distilled water, shaken by hand, set aside, and the first washings removed in the same manner as before. This process was repeated. The soil was then ready for treatment with the alkaline solvent.

The bottles used for this purpose, and for the extractions, were wide necked, with a capacity of about 250 c.c. The bottles with their respective stoppers were all numbered and tared, so that the weight of liquid left with the soil could be ascertained. In the subsequent extractions the total volume of solvent could thus be accurately adjusted.

Alkaline extraction.

Choice of the alkaline solvent.

Russell and Prescott (3) have directed attention to the dark-coloured soil extracts which are produced by solutions of certain salts. This suggested the use of an alkaline solvent containing a salt, as a means of facilitating the solution of the soil organic matter.

Various combinations of salt and alkali were tried, and the conditions of working were made as constant as possible. Solvent power was gauged by the tint of the extract.

In this preliminary work large samples of soil were given various preliminary treatments, viz. untreated, sulphuric acid, hydrochloric acid, nitric acid, on the lines indicated above. The soils were then dried, ground in a mortar and sifted. Portions of 5 gm. each were then stirred with 20 c.c. of the various alkaline solvents, set aside for a short time,

filtered, and the tints of the extracts compared. From the result it was evident that untreated soil, and soil treated with sulphuric acid, was less suitable for the purpose in view than soil treated with hydrochloric acid. Caustic soda and caustic potash were about equal in solvent power, and both were much superior to ammonium hydroxide of equivalent strength ($N/2$). The presence of alkali sulphates or chlorides was not greatly detrimental to the extraction of the coloured bodies. Calcium salts depressed the extraction, but this effect was overcome by the addition of salts which result in the formation of insoluble calcium compounds, notably sodium silicate.

As a result of the above experiments the composition of the stock alkaline solvent used in all the subsequent work was as follows:

Sodium hydroxide (commercial)	44	gm.
Potassium hydroxide	2·2	,,
Sodium phosphate (Na_2HPO_4)	2	,,
Sodium silicate. Solution, sp. gr. 1·12... ...	20	c.c.
Water, to make	1000	,,

After a batch of several litres of the solvent had been prepared, it was left for one or two days to clarify, and the clear liquid was syphoned off and preserved in a full bottle to minimise atmospheric contamination. For use, it was diluted with an equal volume of water, thus making approximately $N/2$ alkali.

Rate of solution of the coloured organic matter.

A portion of soil was shaken with the diluted alkaline solvent, and portions were removed at intervals of 30 minutes in order to observe the tint of the extract. The process of solution of the coloured bodies was found to occur very rapidly during the first 30 minutes, after which it slowed down considerably. In another trial, 10 gm. of soil were mixed with 200 c.c. of the diluted alkaline solvent and shaken for 4 hours. After settling, the extract was removed as completely as possible. The residue was then similarly treated with successive portions of fresh solvent. The tint values of these extracts were:

	After HCl treatment	Untreated
1st extract	4366	2578
2nd ,,	1128	1252
3rd ,,	367	457
4th ,,	352	497

Four hours' shaking was deemed sufficient to extract the major part of the coloured matter soluble in cold alkali.

Extraction of organic carbon from Rothamsted soils.

The preliminary work described above was carried out with soil from the surface 9 in. of Barnfield, Plot 10. The soils used for the main investigation consisted of representative samples of the surface (0–9 in.) and subsoil (9–18 in.) from the plots mentioned in the earlier part of this paper. The air-dried, fine earth (3 mm.) was ground until it would all pass a 1 mm. sieve. The amount of soil taken for each determination represented 10 gm. of oven-dried soil.

The method of extraction may be illustrated by taking Barnfield, Plot 1 C, surface soil as an example.

Ten wide necked, 250 c.c. bottles were fitted with rubber stoppers, numbered and tared. 10·42 gm. of the soil (representing 10 gm. of soil dried at 100° C.) were placed in each bottle, and 21 c.c. of $N/2$ HCl added. The bottles were shaken gently by hand, the stoppers were inserted and tied down, and the bottles were shaken continuously for 2 hours in a mechanical end-over-end shaker. The subsequent removal of the acid extract and washing were carried out as already described. The bottles containing moist washed soil were then weighed, and the weight of residual wash water was thus ascertained. Bottle No. 1 was put aside for determination of total carbon.

Water was next added to each of the other bottles in such quantity that, together with the residual wash water, it made up 100 c.c. 100 c.c. of the stock alkaline solvent were then added. The bottles were then closed and weighed in order to verify that equal amounts of solvent were present in all.

The bottles were dealt with as follows:

No. 1 determined total carbon in the acid-treated soil.

„ 2	determined carbon extracted in 4 hours		by continued shaking with the cold solvent in an end-over-end shaker.
„ 3	„	„ 8 „	
„ 4	„	„ 16 „	
„ 5	„	„ 3 „	by heating the bottles neck deep in a boiling water bath with occasional shaking by hand.
„ 6	„	„ 5 „	
„ 7	„	„ 7 „	

The remaining bottles served as duplicates or spares.

The bottles were put aside for the soil to settle, and 100 c.c. portions of the clear extract were pipetted off for analysis. The determinations of carbon were carried out by the wet combustion method described in the appendix to this paper.

Results.

Table III shows the results of the carbon determination on the Barnfield soils and their extracts.

Table III. *Carbon content of Barnfield soils and their alkaline extracts. Values expressed as percentages of the oven-dried soils.*

	Plot 1 C	Plot 8 O	Plot 8 A
Surface soils			
Acid treated soil	2·85	0·81	0·84
Alkaline extracts:			
4 hours' cold extraction	0·69	0·23	0·29
8 „ „ „	0·82	0·23	0·30
16 „ „ „	0·93	0·26	0·32
3 „ hot „	1·90	0·53	0·59
5 „ „ „	1·94	0·56	0·60
7 „ „ „	1·99	—	—
Subsoils			
Acid treated subsoil	1·02	0·55	—
Alkaline extracts:			
4 hours' cold extraction	0·35	—	—
8 „ „ „	—	0·20	—
16 „ „ „	0·39	0·21	—
3 „ hot „	0·75	0·40	—
5 „ „ „	0·76	0·40	—

The corresponding results for Broadbalk soils are shown in Table IV.

Table IV. *Carbon content of Broadbalk soils and their alkaline extracts. Values expressed as percentages of the oven-dried soils.*

	Plot 2 B	Plot 3	Plot 7
Surface soils			
Acid treated soil	2·91	0·89	1·10
Alkaline extracts:			
4 hours' cold extraction	0·86	0·28	0·40
8 „ „ „	0·95	0·32	0·43
16 „ „ „	1·08	0·33	0·47
3 „ hot „	2·24	0·69	0·85
5 „ „ „	2·26	0·70	0·86
Subsoils			
Acid treated subsoil	1·03	0·62	0·64
Alkaline extracts:			
4 hours' cold extraction	0·43	0·27	0·29
8 „ „ „	0·47	0·27	—
16 „ „ „	0·49	0·31	0·31
3 „ hot „	0·88	0·47	0·50
5 „ „ „	0·87	0·49	0·55

Discussion of results of carbon determinations on soils and extracts.

For both Broadbalk and Barnfield soils, the values for the soils themselves and for the extracts are very markedly higher in the case of the plots which receive annual dressings of dung, as would be expected. It is noteworthy that this difference, which is most pronounced with the surface soils, is also evident with the subsoils although these are below the level of cultivation. A part of this excess of carbon in the subsoil might be due to the decomposition of the residues of roots which penetrate below the cultivation level. In this connection, however, it should be noted that:

(1) The excess of carbon in the subsoil of the dunged plot over that in the subsoil of the unmanured plot is no greater on Broadbalk Field carrying wheat, than on Barnfield carrying mangolds.

(2) The subsoil of the artificial manure plot of Broadbalk (Plot 7) is much poorer in carbon than that of the dunged plot, and is, in fact, scarcely any richer than that of the unmanured plot (Plot 3), although the artificial manure plot produces a yield which is as high as that on the dunged plot and, therefore, presumably contributes a similar amount of root residues to the subsoil.

These facts appear to indicate that the excess of organic matter in the subsoil of the dunged plots is not derived to any appreciable extent from the decomposition *in situ* of an excess of root residues in these subsoils, but rather, that organic matter is carried down to the subsoil from the surface soil. This view is supported by the fact that, as shown below, the organic matter of the subsoil is more soluble than that of the surface soil.

The relative behaviour of the organic matter of the soils and subsoils from the different plots, with regard to solubility in alkali, is best brought out by expressing the results in Tables III and IV as percentages of the total amount of carbon in the soils themselves. The results are expressed in this way in Table V (p. 467).

It is evident from these results that the organic matter of these soils is extracted in two stages. The greater part of the carbon soluble in cold alkali is extracted in 4 hours, and prolongation of the extraction to 8 or 16 hours produces only a relatively small increase. Extraction with hot alkali, however, brings into solution about twice as much carbon as that extracted in the cold, and here prolongation of the hot extraction beyond 3 hours produces practically no further increase.

These results appear to indicate that the organic matter of these soils is present in three forms, one readily soluble in cold alkali, another only slowly soluble in cold dilute alkali but rapidly soluble in hot alkali, and a third which is insoluble in hot dilute alkali. Odén regards humin, which is insoluble in cold alkali, as the anhydride of humic acid(4). The above results are explicable, according to this view, if we suppose that the humic acid is extracted by cold alkali, and that the part which is readily soluble only in hot alkali consists largely of humin, which has first to undergo hydration to humic acid before it comes into solution as sodium humate.

Table V. *Amounts of carbon in soil extracts, as percentages of the total carbon content of the acid-treated soils before extraction.*

	Barnfield					Broadbalk field					
	Plot 1 C		Plot 8 O		Plot 8 A	Plot 2 B		Plot 3		Plot 7	
Alkaline extracts	Surface	Subsoil	Surface	Subsoil	Surface	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil
4 hours' cold	24	34	28	—	34	29	41	31	43	36	46
8 „ „	28	—	28	37	35	33	45	36	43	39	—
16 „ „	32	38	31	38	38	37	47	37	50	43	48
3 hours' hot	66	73	65	73	70	77	84	77	76	77	79
5 „ „	68	74	69	73	71	77	83	78	79	77	85

A comparison of the value for any soil with that for its corresponding subsoil shows that the carbon in the subsoil is uniformly more soluble than that in the surface soil, and that this difference is chiefly due to an increase in the part soluble in cold alkali. This supports the view, referred to above, that a large part of the organic matter in the subsoil has been brought down in solution from the surface soil.

Although certain minor differences are apparent between the results from the different plots, the most striking point is the great general similarity in the results, despite the great differences between the treatments which the plots have received. Thus cold alkali extracts practically identical proportions of the total carbon from the surface soil of the dunged plot of Barnfield (1 C), and the unmanured plot (8 O). This is also the case with hot alkali. The same is true for the corresponding plots of Broadbalk (2 B and 3). In the case of the artificial manure plots the proportion of carbon soluble in cold alkali is slightly higher, but with hot alkali the proportion extracted is not sensibly different from that extracted from the dunged or unmanured plots. Although, as pointed out above, the carbon of the subsoils is somewhat more soluble than that of the surface soils, when compared among themselves the

results for the subsoils of each field show the same close similarity as those for the surface soils. The values for the Broadbalk plots are throughout somewhat higher than those for the corresponding Barnfield plots, a fact which is presumably in some way connected with the different cultural treatment of the two fields.

The view that the organic matter insoluble in cold alkali but soluble in hot consists largely of humin, which undergoes hydration to humic acid before it becomes alkali-soluble, is supported by the results of the following experiment. The preliminary acid-treatment of soil from Plot 1 C and subsoil from Plot 8 O on Barnfield was carried out for 2 hours at the temperature of the boiling water bath, instead of in the cold. The alkali extractions were carried out as before. The results are compared with those for the soils treated with cold acid in Table VI.

Table VI. *Effect of hot acid treatment on alkali solubility of soil organic matter. Barnfield soil.*

Carbon in alkali extract, as percentage of total carbon.

	Plot 1 C surface		Plot 8 O subsoil	
Alkali extraction	Treated with *hot* acid	Treated with *cold* acid	Treated with *hot* acid	Treated with *cold* acid
4 hours' cold	32	24	44	—
8 „ „	35	28	46	37
3 hours' hot	69	66	72	73
5 „ „	71	68	—	73

Treatment with hot acid has caused a marked increase in the solubility of the organic matter in cold alkali, but the total amount dissolved in hot alkali is about the same, whether hot or cold acid was used. This may be explained on the assumption that treatment with hot dilute acid has caused the hydration of a part of the humin into humic acid.

The tints of the alkaline extracts.

All the results so far described in this paper refer to the soluble organic matter of the soils in question in terms of carbon, without any attempt to discriminate between the different kinds of organic compounds in which that carbon may occur. It is known that a great variety of organic compounds may occur in the alkaline extracts of soils—Schreiner, Shorey and their co-workers isolated more than 16 different compounds from such extracts(5). The quantitative determination of even a few of these compounds in alkaline extracts from the soils dealt with in this paper would be a task of considerable magnitude,

and would necessitate the development of a complex and specialised analytical technique.

There is, however, one characteristic of the humic matter of soil which readily lends itself to measurement—its colour. During the process of humification, colourless organic materials are converted to strongly coloured humic matter; the subsequent breakdown of humic matter may also be attended by the production of weakly coloured or colourless substances. The intensity of the colour of the organic matter in an alkaline extract of soil may, therefore, be regarded as an indication of the proportion of humic matter in that organic matter.

Measurements were, therefore, made of the tints of the alkaline soil extracts of which the carbon content has already been recorded. The shades of colour were very similar in all these extracts, so that reasonably accurate matches could be obtained in a colorimeter. The solutions used consisted of the actual extracts used as already described for carbon determination, which had been allowed to clear completely by settling. The extract obtained by 5 hours' treatment with hot alkali was used as the standard of comparison for each soil, all the other extracts from that soil being compared with it. The final matching in the colorimeter was carried out with solutions which had been adjusted by dilution where necessary until they did not differ by more than 20 per cent. in tint. The ratio of tint to carbon content is arbitrarily fixed as unity for the 5 hours' hot alkali extract in the results quoted below, so that the variations in the tints of the organic matter of the other extracts may be judged by the degree of variation of their tint/carbon ratios from unity.[1] The results are shown in Table VII (p. 470).

In the case of the Barnfield surface soils it is seen that there is, on the whole, little variation in the values for the different extracts from any one soil, indicating that the tint of the readily soluble organic-carbon (soluble in cold alkali) is not greatly different from that of the organic carbon, soluble in hot alkali. In the case of the two Barnfield subsoils, however, the readily soluble organic carbon has a weaker colour intensity

[1] These tint/carbon ratios were actually calculated as follows:

If the 5 hours' hot extract, containing x per cent. of carbon, in a column a mm. high, was matched in the colorimeter by a column b mm. high of another extract containing y per cent. of carbon, then the tint/carbon ratio of the second extract, in terms of that of the 5 hours' hot extract as unity, is

$$\frac{a}{y} \div \frac{b}{x} = \frac{ax}{by},$$

since the tints of the solutions are inversely proportional to their heights when matched in the colorimeter.

than that soluble in hot alkali. The results for the Broadbalk soils are less regular. The readily soluble organic carbon from the dunged plot (2 B) is not very different in tint from the organic carbon soluble in hot alkali, but in the case of the other two surface soils, the readily soluble carbon has a higher colour-intensity than that soluble in hot alkali. The same is true of the subsoil from the dunged plot, but in the case of the subsoil from the other two plots the values do not show much variation.

Table VII. *Tint/carbon ratios of Barnfield and Broadbalk soil extracts.*

The tint/carbon ratio of the 5 hours' hot extract is in each case expressed as unity.

	Barnfield						Broadbalk					
	Plot 1 C		Plot 8 O		Plot 8 A		Plot 2 B		Plot 3		Plot 7	
Alkaline extracts	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil
4 hours' cold	0·97	0·69	0·91	—	0·90	—	0·92	1·23	—	0·93	1·13	1·03
8 „ „	0·85	—	0·96	0·80	0·93	—	0·94	1·19	1·22	1·00	1·09	—
16 „ „	0·84	0·69	1·00	0·76	0·91	—	0·91	1·16	1·15	0·84	1·13	1·06
3 „ hot	0·97	0·95	1·04	0·95	1·00	—	1·00	1·00	1·03	1·02	1·02	1·10
5 „ „	1·00	1·00	1·00	1·00	1·00	—	1·00	1·00	1·00	1·00	1·00	1·00

The above results do not enable a comparison to be made between the colour-intensities of the organic carbon of the different plots. To do this, it is necessary to compare with one another the tints of the extracts from the different soils and subsoils. For this purpose fresh extracts were prepared by treatment for 16 hours in the cold and 5 hours in the hot, all the soils being treated simultaneously under identical conditions. The extracts were set aside for 48 hours, cleared by centrifuging and compared colorimetrically. From the results obtained, in conjunction with the carbon contents of the extracts, tint/carbon ratios were calculated as before, the standard of comparison in this case being the 5 hours'

Table VIII. *Tint/carbon ratios of Barnfield and Broadbalk soil extracts.*

Referred to that of Barnfield, Plot 8 O, subsoil hot extract expressed as unity.

	Barnfield						Broadbalk					
	Plot 1 C		Plot 8 O		Plot 8 A		Plot 2 B		Plot 3		Plot 7	
Alkaline extract	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil	Surface	Subsoil
16 hours' cold	1·95	1·45	1·49	0·98	1·55	—	1·98	1·56	1·76	1·14	1·65	1·30
5 hours' hot	2·02	1·60	1·43	1·00	1·49	—	2·00	1·57	1·12	1·15	1·66	0·98

hot extract from Barnfield, Plot 8 O subsoil, for which the tint/carbon ratio was expressed as unity. The values obtained are shown in Table VIII.

Three points are clearly seen from these figures:

(1) The organic carbon extracted from the surface soils is more deeply coloured than that of the corresponding subsoils.

(2) The organic carbon is most deeply coloured in the extracts from the surface soils which receive annually heavy dressings of dung (Plots 1 C and 2 B).

(3) The organic carbon in the extracts from the subsoils of the plots receiving no dung or artificial fertilisers is least strongly coloured of all.

Too great importance must not be attached to the values for the hot extracts, since it has been found that the tint of the extracts diminishes somewhat with heating, the loss falling relatively most heavily on the weakest coloured solutions. Some of the apparent anomalies in Table VII may be due to this cause, but not the *higher* values given by the hot extracts of the Barnfield subsoils compared with the cold extracts. In Table VIII, however, most of the results show a reasonably good agreement between the values for the cold and hot extracts. The cold extracts in any case are free from this objection, and the variations in the tint/carbon ratio in Table VIII for these extracts, from which the above conclusions have been drawn, may be interpreted on the assumption that the organic matter in the extracts is of two types, colourless and coloured.

Actually, of course, it is possible that there is a series of substances with tints ranging gradually from entirely colourless, through increasing degrees of colour-intensity to the most strongly coloured bodies. However, in the present state of our knowledge of the nature of the organic substances of soils, and in the absence of reliable methods for their separation and characterisation, it is not possible to interpret the tint/carbon ratio of the organic matter of a given soil from this point of view. But if we select a standard preparation of strongly coloured humic matter, and compare the soil extracts with it colorimetrically, we can calculate the relative proportions of the organic carbon which would have to be present in the form of colourless non-humic organic matter, and in the form of the coloured standard humic material, to give the tint/carbon ratio obtained for the extract in question. Although this procedure is essentially an empirical one, it affords a basis of comparison of the different extracts, from which we can express the degree of humification of the organic matter of that extract in terms of the standard humic material.

The material that was selected for this purpose was the preparation made by Merck, under the name of "Acidum Huminicum". Although this is by no means a "pure" material, it is readily procurable, and is a typical humic preparation. The carbon content of this material, dried at 100° C., was 53·79 per cent. Solutions of this material were made in $N/2$ caustic soda, of strengths comparable with the cold and hot soil extracts, and compared in the colorimeter with the same standard as used above (Barnfield, Plot 8 O, subsoil 5 hours' hot extract). When the "acidum huminicum" was dissolved in cold alkali, the tint/carbon ratio, on the same basis as that used in Table VIII, was 4·92, whilst after the solution had been kept at 100° C. for 5 hours the tint/carbon ratio was 4·56, the heating having caused a certain loss of colour. It will be seen that the tint/carbon ratio of the "acidum huminicum" is much higher than that of any of the soil extracts.

From the tint/carbon ratios of the 16 hours' cold extracts of the soils, and the value for the cold alkaline solution of "acidum huminicum", the proportions of the total carbon in these extracts which would be present in the form of colourless non-humic substances have been calculated with the following results (Table IX):

Table IX. *Calculated percentages of total carbon present in the form of colourless substances.*

16 hours' cold alkali extracts.

	Barnfield			Broadbalk		
	Plot 1 C	Plot 8 O	Plot 8 A	Plot 2 B	Plot 3	Plot 7
Surface soil	60	70	69	60	64	66
Subsoil	69	80	—	69	77	74

The basis of these figures is, of course, empirical. They indicate the percentage of the total carbon which would be present in the colourless form if the whole of the coloured material were present in the form of "acidum huminicum". Whilst these results emphasise the need for further work with a view to separating the coloured and the colourless constituents of the organic matter in alkaline soil extracts, it must be remembered that "acidum huminicum" is a preparation from peat, and that possibly the coloured humic matter of mineral soils is intrinsically less strongly coloured than that of peat. If this is so, the calculated values in Table IX may be greatly in excess of the actual percentage of non-humic, colourless organic matter in the extracts.

APPENDIX.

The determination of organic-carbon in soils and soil extracts.

For the determination of the carbon content of alkaline extracts of soils, only a wet combustion method is suitable, in which the soils or extracts are oxidised by a mixture of chromic and sulphuric acids, and the carbon dioxide produced is absorbed by caustic soda in a Reiset tower. Carbonated alkali is then determined by titration with a standard acid, using first phenolphthalein then methyl orange as indicators. A tube of hot copper oxide was introduced between the combustion flask and the absorption tower, in order to complete the oxidation of any carbon compounds which had escaped oxidation in the chromic acid mixture.

This method was modified as follows:

A detector was used whereby the gas stream could be diverted past an exposed drop of baryta, in order to show when a combustion was complete, and also whether the air purifying system was efficient.

A **U**-tube containing baryta, beyond the Reiset tower, showed that the Reiset tower was inefficient when a large rush of carbon dioxide occurred, as, for instance, in the first period of a combustion. A modified absorption tower was devised to meet this contingency.

The oxidation of soils with the chromic-sulphuric acid mixture was effected in a Kjeldahl flask, while a current of purified air was drawn through the flask.

The soil to be oxidised was placed in a Kjeldahl flask, and 10 c.c. of the chromic acid mixture was introduced by means of the dropping funnel passing through one hole of the doubly bored rubber stopper. The bore of the funnel was washed out with a few drops of water, after which 50 c.c. of strong sulphuric acid were introduced, and heat was gently applied.

For the oxidation of alkaline extracts of soils it was essential to concentrate them. By concentration at atmospheric pressure, it was difficult to avoid the absorption of appreciable amounts of carbon dioxide from the air. This was, however, avoided by evaporation under reduced pressure.

The extract was concentrated in the Kjeldahl flask, in which the oxidation with chromic acid was to be subsequently carried out. When the extract was sufficiently concentrated, air was gradually admitted through a soda lime tube and the flask was left to cool, after which the extract was ready for combustion. When cold, the concentrated extract

occupied about 5 c.c., as a paste, or less as a brown solid. The combustion was carried out in the same manner as for soils. For the combustion the current of air entered through the dropping funnel to the lower part of the combustion flask. The end of the dropping funnel should neither be constricted nor turned up, as these promote blocking. Acid vapours from the combustion flask were largely condensed and returned by a reflux condenser. All that escaped condensation passed directly into a silica combustion tube containing granular copper oxide between copper gauze plugs. This tube was maintained at a dull red heat throughout, and served to oxidise completely any volatile carbon compounds which had passed from the combustion flask. Chlorine was largely arrested by the copper spirals. The gases then passed to the carbon dioxide detector, and thence through a suspension of silver sulphate to remove any chlorine which had passed the copper spirals. The purified gas passed thence into the absorption tower.

In the later part of the work described in this paper, carbon dioxide was estimated gravimetrically. The only additional purification necessary was the removal of moisture, which was effected by the insertion of a drying tube filled with pumice fragments and concentrated sulphuric acid.

The carbon dioxide detector was placed in a by-pass between the silica combustion tube and the silver sulphate tube. This consisted of a U-tube with side tubes, the gases entering by one arm and leaving by the other. Both limbs of the U-tube were closed by rubber stoppers, one of which carried a dropping funnel, the end of which was drawn out to a fine point, so that a very small drop of baryta solution could hang suspended immediately in the path of the gases entering by the side arm. The drop could be renewed from the funnel, and the carbonated drop drawn out through a stopcock at the base of the U-tube. The appearance of the contents of the combustion flask gave a fair indication of the progress of a combustion, and after a suitable time the gas stream was examined for carbon dioxide by diverting it past the baryta. At the first indication of turbidity in the drop of baryta, the direct path was resumed. This device was also used before starting a combustion, to ascertain whether the joints were good and the purifying tubes efficient.

The combustions were carried out as follows:

The combustion tube was heated, and a current of air was drawn through the apparatus with an empty Kjeldahl flask in position until no carbon dioxide could be detected in the stream. Suction was then stopped, and when the pressure had equalised, the Kjeldahl flask containing the soil or the concentrated extract was then substituted

for the empty Kjeldahl flask and the carbon dioxide absorption tubes were connected. The oxidising mixture was then added and as soon as the first rush of gas had slowed down, heat was applied, and the contents of the flask maintained at the boil until the completion of the combustion.

The absorption of carbon dioxide in caustic soda in a Reiset tower, for the volumetric determination, was found to be incomplete during the first rush of gas, and other forms of tower were investigated.

A special tower was devised, consisting of a tube 60 cm. long and 1·7 cm. bore, in which 50 c.c. of alkali occupied a length of 21 cm. A bored rubber stopper was fitted around the lower end, and to it was vulcanised a stout disc of rubber.[1] A small glass tube, closed at the base and with one hole drawn in at the side, was fitted over the stopper and served to catch drops. The tower was held upright in a small filter flask which was partly filled with sulphuric acid so as to reduce the gas capacity of the apparatus. A large number of holes was pierced in the rubber disc with a pin, and it was found that when suction was applied, an exceedingly fine mist of bubbles was produced in the alkali, which caused it to fill up 35 cm. to 40 cm. of the tube. Under these circumstances perfect absorption of carbon dioxide was invariably achieved. A fine spray was thrown up for 4 cm. to 8 cm. above the surface of the alkali. To prevent the escape of this, a ball of paraffin wax was dropped into the tower, and this was subsequently poured into the titration flask with the alkali. It was possible to wash out this tower, the rubber foot, and the "drop tube" without unduly diluting the alkali. Owing to the smallness of the holes in the rubber, the tower could be disconnected from the flask without more than a drop of the alkali escaping into the "drop tube".

In order to obtain the best possible end points in the double titration it was necessary to use "A.R." caustic soda. It is desirable to keep the volume of the liquid as small as possible during the titration; this was accomplished by the use of $4N$ sulphuric acid until the first stage of neutralisation was nearing completion. After this point, $N/2$ acid was used.

Although the conditions worked out for this double titration by Browne and Escombe were rigidly observed, the sharpness of the end points was not all that could be desired; it was found possible to improve this by certain modifications, but in spite of this, the accuracy of the

[1] The most suitable material was that obtained from the inner tube of a motor-car tyre, about 2 mm. thick.

method was less than that obtained by gravimetric determination of the carbon dioxide, and this method was used in the later stages of the work.

For this method, the best form of absorption tube to cope with the violent initial rush of gas was found to consist of a simple tube, made by drawing out ordinary wide tubing of 28 mm. bore. Such tubes had an effective length of 19 cm. and contained in order, from the drawn-out end, cotton wool; glass wool; phosphorus pentoxide 3·5 cm.; glass wool; a disc of wire gauze; cotton wool; soda lime 12 cm.; cotton wool.

One such tube, and one U-tube packed with the same chemicals, were used in tandem for each combustion. It was not unusual to find no change in the weight of the U-tube. Two similar tubes were used as counterpoises during the weighing, and alternately as absorption tubes. With this form of tube it is very easy to rub off the moisture film as there are no irregularities of surface and no taps. As the results obtained with Broadbalk soils by the gravimetric method showed certain differences from those obtained with Barnfield soils by the volumetric method, carbon was redetermined in some of the Barnfield soils by the gravimetric method to ascertain whether the differences could be accounted for by difference of method, or whether they were real.

The following comparative figures refer to 10 gm. soil.

Barnfield. Plot 1 *C. Surface soil.*

		gm.
Total carbon	Volumetric method	0·285
Total carbon	Gravimetric method	0·288

Barnfield. Plot 1 *C. Subsoil.*

		gm.
Total carbon	Volumetric method	0·102
Total carbon	Gravimetric method	0·106

Barnfield. Plot 8 *O. Surface soil.*

		gm.
Total carbon	Volumetric method	0·081
Total carbon	Gravimetric method	0·086

Barnfield. Plot 8 *O. Subsoil.*

		gm.
Total carbon	Volumetric method	0·055
Total carbon	Gravimetric method	0·055

Although the results from the gravimetric method are generally slightly higher, the differences are small and insufficient to affect the comparative value of the results obtained by the volumetric method.

Summary.

The alkali extraction of the organic matter from the soils of certain plots of the classical permanent experiments on Barnfield and Broadbalk at Rothamsted, receiving respectively organic manure, artificial fertilisers and no manurial treatment, has been studied, together with a colorimetric examination of the extracts for the comparison of the content of humic matter.

It is shown that, in spite of the different cultural and manurial treatments which the different plots have received, there is a marked similarity in the properties of the organic matter of these soils, with regard to its behaviour on extraction with cold and hot dilute caustic soda, and the colour intensity of the organic matter in the extracts.

The methods used for the determination of organic carbon in the soils and their extracts are noted in an Appendix.

The results discussed in this paper are further considered, along with those in the two next following Parts (III and IV), in Part V of this series, in their bearings on the origin of the humic matter of the soil.

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(*Received March 3rd*, 1930.)

STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

III. THE FORMATION OF NATURAL HUMIC MATTER.

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The investigations on the alkali extraction of organic matter from Rothamsted soils, which are dealt with in the previous paper of this series (1), revealed a marked similarity in the character of the soil organic matter of different plots, so far as solubility in weak caustic alkali is concerned, in spite of the fact that these different plots had received organic matter of widely different nature and composition. This tendency of the organic matter of the soil to possess its own special and tolerably uniform properties, no matter what its origin, has given rise to various hypotheses, according to which the characteristic constituent of that organic matter—the humic matter—is derived only from one parent substance or class of substances present in plant materials. The investigations described in this and the succeeding paper were concerned with the formation and properties of humic matter, of both natural and artificial origin, with the object of testing the validity of the more important of these hypotheses, and, if possible, indicating the true precursor of soil humic matter. These investigations may be divided into two groups:

(1) A quantitative study of the "humification" of various plant materials and of their purified constituents under controlled conditions comparable with those obtaining in nature, in the hope of being able to correlate humic matter formation with the initial presence and subsequent disappearance of one characteristic plant constituent.

The investigations in this group are dealt with in this paper.

(2) A comparison of the products of various chemical reactions giving rise to artificial humic matter, with preparations of natural humic matter. In addition, two of the reactions giving rise to artificial humic matter—the action of mineral acids on furfurol and on ω-hydroxy-

[1] One of the authors (M. M. S. du Toit) was awarded the degree of Doctor of Philosophy by the University of Cambridge in 1924, for a Thesis embodying the results described in this paper.

[2] The investigations dealt with in this series of papers were carried out by or under the direction of the senior author (H. J. P.) up to the time of his leaving the Rothamsted Experimental Station in 1927.

methyl furfurol, and the interaction of hexoses and amino-acids—were selected for special study.

The investigations in this second group are dealt with in the next paper of this series.

QUANTITATIVE STUDY OF THE HUMIFICATION OF VARIOUS PLANT MATERIALS.

The main organic constituents of plants may be broadly classified as (1) cellulose, (2) other hexosans and hexoses, (3) furfuroids (pentosans, etc.), (4) lignins, (5) proteins. Although fats are important constituents of some plant materials, they are of minor occurrence in the materials from which humic matter is commonly formed.

Materials were chosen, containing these five groups in differing proportions, and their composition in regard to these groups was determined. They were then allowed to rot down under controlled conditions and, at intervals during their decomposition, samples were taken and analysed as before, whilst in addition, the content of humic matter was determined. From a consideration of the results obtained, an attempt was made to correlate the amount of humic matter formed with the extent of the disappearance of the various constituents of the original materials.

Other experiments were carried out under the same conditions but working with purified preparations of some of the chief plant constituents, *i.e.* cellulose, starch, a hexose, a pentosan, a pentose, lignin and a protein. These experiments would indicate which of these constituents could give rise to humic products under conditions of rotting comparable with natural conditions, and thus had a direct bearing on the results of the work on the plant materials themselves.

Incidentally the part played by micro-organisms was also studied.

EXPERIMENTAL.

(1) *Methods of analysis.*

In determining the proportions of substances belonging to the five broad groups mentioned above in the plant materials used in these experiments, no attempt was made to determine individual chemical substances or more closely related classes of substances, but only the amounts in each broad group as a whole, it being also understood that these groups cannot all be regarded as clearly defined. They are, however, sufficiently sharply differentiated from one another, to encourage the

hope that the study of the fate of the different groups as a whole during humification might indicate to which group the parent substance of humic matter—if *one* parent substance there be—belongs. The way would then be clear to a closer study of the relation of individual members of that group to humic matter formation.

The determinations made on the original plant materials and on the products resulting from their partial humification were:

(*a*) *Furfuroids.* The standard method of Krober and Tollens(2) was used, in which the furfurol produced by distillation of the material with 12 per cent. hydrochloric acid under specified conditions is determined as the phloroglucide, the result being expressed as pentosan.

(*b*) *Lignins.* Willstätter and Zechmeister's(3) method was used. About 2 gm. of the dried, finely powdered material was treated with 50 c.c. of 42 per cent. hydrochloric acid, left overnight in a stoppered flask, and the residue collected, washed, and weighed after drying at 100° C. Since humic matter is also insoluble under these conditions, it is necessary, when dealing with humified materials, to determine the amount of humic matter in the residue, by a method described later in this paper (see p. 484).

(*c*) *Crude protein.* This was determined in the conventional manner, *i.e.* total nitrogen content multiplied by 6·25.

(*d*) *Non-cellulosic hexosans and hexoses.* The residual liquid left after boiling with 12 per cent. hydrochloric acid for the furfuroid determination (*a*) was neutralised and titrated against Fehling's solution, the result being expressed as glucose. Experiment showed that starch could be quantitatively accounted for under these conditions. The result of this determination is an approximate measure of the amount of hexoses and of more readily hydrolysable hexosans and hexose-yielding substances, as distinguished from cellulose, which is almost unaffected by this treatment.

(*e*) *Cellulose.* This was not directly determined, but that part of the organic matter of the original material which was not expressed in the results of the four determinations under (*a*) to (*d*) above was taken as an approximate measure of the cellulose content. Whilst it is not pretended that this is more than a rough approximation, it was considered sufficient for the purpose in view, more especially for comparative purposes, but to avoid misunderstanding the values obtained by difference in this way are shown as "cellulose, etc."

(2) *Plant materials used.*

The original plant materials used for these humification experiments were:

(*a*) Wheat straw, grown at Rothamsted.
(*b*) Clover hay, grown at Rothamsted.
(*c*) Maize cobs, from Potchefstroom, South Africa.
(*d*) Pine sawdust.[1]

All the materials were ground to pass a 1 mm. sieve.

(3) *Humification experiments.*

In each series of experiments the materials, after soaking in water, were mixed with sufficient calcium carbonate and nutrient salts, inoculated with soil extract, water added in just sufficient amount to avoid waterlogging, and the mixture incubated at 32° C. in bottles plugged with cotton wool, and carrying two aeration tubes, one of which passed to the bottom of the bottle (Series I and III), or in beakers covered with pads of cotton wool (Series II). The mixtures were set up in this way, so as to ensure that the subsequent rotting went on under conditions closely comparable with those actually obtaining in the soil. Thus by periodically drawing air through the mixture, efficient aeration was obtained. By inoculating the mixtures with an extract of soil and by the addition of nutrient salts, the presence and the activity of soil organisms was ensured. By the addition of calcium carbonate, the mixtures were prevented from becoming acid during rotting. The mixtures were incubated at 32° C. so as to accelerate the rate of rotting, and thus to make it possible to obtain measurable results in a reasonable time. The bottles or beakers were examined at regular intervals, moisture adjusted to the right degree, and the materials well stirred up. The moisture conditions in all cases were kept as nearly identical as possible, although the actual amounts of water in the various mixtures differed considerably, owing to different moisture absorbing capacities of the materials used.

Samples of the mixtures were taken initially and at intervals during their decomposition, for analysis as above, and also for determination of loss on ignition of the oven-dried material, as a measure of the percentage of total organic matter. All the determinations, after being calculated on the ash-free residual organic matter, were expressed as

[1] Contrary to expectation, it was found that pine sawdust, which had been extracted with alcohol and ether with the object of removing resins, rotted down under the conditions described less readily than before such treatment, so that the sawdust was used in the untreated condition.

percentages of the ash-free organic matter initially present, a measure of the loss of total original organic matter during the decomposition being obtained from the value of the ash content at the time of sampling in comparison with that initially. Special care was taken, by repeated sub-sampling of the whole mixture at each time of sampling, to obtain samples which were truly representative.

(*a*) *Series I.*

In this series the four materials named above were used, with the admixture of 250 gm. of fine sand to 30 gm. of plant material. The nutrient salts added, per 30 gm. of plant material, were:

Calcium carbonate	15 gm.
Ammonium carbonate (15 per cent. N)	2·5 gm.
Ground mineral phosphate (Gafsa) ...	2 gm.

The clover mixture soon began to turn black, whilst that containing straw became brown; the other mixtures darkened to a lesser degree. After three weeks the clover mixture had become a sticky black rotting mass, the others having rotted to a much lesser extent, and in the order: straw, maize cobs, sawdust.

(*b*) *Series II.*

In this series, sawdust was omitted. The materials were allowed to rot without the admixture of sand. The degree of grinding was much finer, the materials being reduced to the condition of a flour. The nutrient salts added were:

Calcium carbonate	5 per cent. of dry weight of plant material
Dipotassium phosphate	5 ,, ,, ,,
Ammonium carbonate, to give N	3 ,, ,, ,,

The materials were stirred up *in situ* with a glass rod, and the moisture content adjusted daily. The action was extremely rapid in this case, the materials having reached a fairly advanced stage of rotting after a week. After three weeks the clover had assumed the appearance and odour of a good bullock dung, and straw that of farmyard manure. The maize cob meal had blackened considerably, and in all cases this occurred particularly on the surfaces exposed to the air. The materials were tested continually to see that acid conditions did not arise. The vessels were weighed after a month, and the amount of dry matter determined. It was found that the clover had lost 64, straw 36 and cobs 44 per cent. At the end of two months the experiment was stopped, and the rotted

materials air-dried and ground for analysis, after weighing the beakers to determine total loss in dry matter over the whole period.

(*c*) *Series III.*

This series differed from the preceding ones in the fact that the materials used were, in this case, purified preparations of individual plant constituents instead of natural plant materials themselves. The substances used were: (i) *Lignin* prepared from wheat straw by the method of Beckmann, Liesche and Lehmann(4). The preparation was a fine yellow powder, entirely soluble in caustic soda, and free from furfuroids. (ii) *Cellulose*, Whatman No. 41 filter paper, pulped and dried. (iii) *Xylan*, prepared by Heuser's modification of Salkowski's method(5). The preparation was a pale yellow powder, soluble in soda, and without action on Fehling's solution. (iv) *Xylose*, prepared from maize cobs by acid hydrolysis, and recrystallised. The product as used contained 92 per cent. pure xylose. (v) *Starch*—pure potato starch. (vi) *Glucose*—"A.R." (vii) *Protein*. Commercial fibrin, from blood, was used, no suitable preparation of vegetable protein being available.

The substances were each mixed with 25 times their weight of fine pure sand, and with 5 and 10 per cent. of their weight of dipotassium phosphate and calcium carbonate respectively, together with 2 per cent. of nitrogen. In most cases the nitrogen was added as ammonium carbonate, but in certain cases it was added as glycine or as fibrin. The mixtures were moistened, and autoclaved at 120° C. for 15 minutes. Two parallel sets were used; one set was inoculated with soil extract after autoclaving, whilst the other remained sterile. All were incubated at 32° C. In the inoculated set a very vigorous fermentation set in, with the sugars, starch, xylose and protein, and less in the case of cellulose. The only change noticeable with lignin was a gradual darkening in colour, but no colour change was noticeable with any of the other substances. With the sterile set no change whatever was discernible except with lignin, which darkened to about the same extent as in the inoculated set. The contents of the bottles were analysed after six months.

(4) *Measurement of degree of humification.*

In the analytical method described under (*b*) above, humic matter remains with the lignin as determined by treatment with 42 per cent. hydrochloric acid. At the time that this work was carried out, no method was known for the quantitative separation of humic matter from lignin, or from mixtures of other plant materials containing lignin. It was,

therefore, necessary to adopt an indirect method for determining the degree of humification of the various materials examined. The characteristic which distinguishes humic matter from plant constituents and their other decomposition products is its colour. A colorimetric method was, therefore, adopted for determining the degree of humification—the method used being Eden's modification of Odén's method (6), in which the tint of a freshly prepared extract of the material with hot strong caustic soda is compared with that of a freshly prepared caustic soda solution of a standard humic acid preparation. That the whole of the humic matter present in a rotted plant material is, in fact, present in the "lignin" fraction obtained by treatment with 42 per cent. hydrochloric acid was shown by the fact that identical results were obtained by the colorimetric method, whether applied to the rotted material as a whole or to the "lignin" fraction. In support of this the following results may be quoted:

Humification value determined on whole material = 4·47 per cent.
„ „ „ on "lignin" fraction = 4·53 „

Since it is not known whether the specific colour intensity of the humic matter in the rotted materials examined is identical with that of the standard preparation of humic matter, used in the colorimetric determination (Merck's "Acidum Huminicum"), the "humification values" derived from such determinations, and quoted in the results given below, are calculated in terms of this preparation, and they indicate the percentage of "acidum huminicum" which would give to the caustic soda extract the same colour intensity as that actually obtained from the materials under examination. Further, in order to allow for the presence of humic matter in the "lignin" fraction as determined by treatment with 42 per cent. hydrochloric acid, the "humification value" is subtracted from that for the "lignin" fraction to give a corrected value for the percentage of lignin in the rotted materials ("lignin value").

(5) *Results obtained.*

The figures in Table I show the amounts of the different groups of substances, determined as described in section (1) above, in the materials studied, before rotting.

The changes in composition of the materials as a result of rotting are shown, for Series I and II, in Table II.

The relative order of rates of rotting in Series I, as measured by the loss of total organic matter, was in descending order: clover, straw, maize cobs, sawdust, *i.e.* in order of protein content, although equal

quantities of extra inorganic nitrogen had been added in each case. The relative rate of rotting was not directly related to the relative content of furfuroids, sugars or cellulosic materials. Thus, *e.g.* clover which contained only 19 per cent. furfuroids rotted much faster than maize cobs, which contained 45 per cent. of this constituent. The physical nature of the material is also an important factor, and this may possibly be correlated with the manner in which the constituents are combined, and with the protein content.

Table I. *Composition of plant materials used.*

Material	Cellulose, etc. (%)		Hexoses and non-cellulosic hexosans as glucose (%)		Furfuroids (%)		Lignin (%)		Crude protein (%)		Ash (%)
	Series I	Series II	Series I	Series II	Series I	Series II	Series I	Series II	Series I	Series II	Series II
Clover hay	38·2	57·0	4·1	7·5	19·5	12·7	21·2	15·7	17·1	4·6	7·3
Wheat straw	36·6	35·5	5·3	11·8	35·5	28·4	19·3	17·3	3·3	7·0	7·0
Maize cobs	37·2	40·8	5·2	14·2	45·4	31·2	10·6	11·8	1·7	5·2	2·0
Sawdust	49·0	—	5·1	—	12·5	—	32·8	—	0·3	0·6	

Table II. *Changes in composition of plant materials on rotting.*

Columns A. Expressed as percentage of the total amount of ash-free organic matter originally present.

Columns B. Expressed as percentage of the amount of the respective constituent originally present.

Material	Loss of: Total ash-free organic matter	Cellulose,* etc.		Furfuroids		"Lignin value"		Humification value
	A	A	B	A	B	A	B	A
Series I:								
Clover hay	54·0	31·2	52·5	16·4	84·1	12·4	58·5	6·2
Wheat straw	49·0	10·3	22·7	32·7	92·2	9·2	48·0	3·2
Maize cobs	36·9	4·3	9·6	31·9	70·2	3·0	28·8	1·1
Sawdust	21·4	9·0	16·4	8·5	67·6	3·9	12·0	0·9
Series II:								
Clover hay	69·6	53·3	82·7	10·0	79·0	11·6	74·0	5·3
Wheat straw	62·8	34·8	73·6	23·9	84·1	6·6	37·9	2·4
Maize cobs	66·8	39·2	71·3	24·3	77·9	5·3	45·1	2·0

* It should be noted that, owing to the difficulty of determining starch and soluble sugars (if any) in the rotted material, the values for loss of "cellulose, etc." include the losses of these constituents.

With regard to the loss in the various constituents, this fell most heavily on furfuroids, which appear to be attacked preferentially to, and possibly to exert a protective action on, the cellulose.

In Series II the relative order of rates of rotting was as in Series I,

but the total amount of organic matter lost was higher, no doubt partly owing to the finer state of subdivision. Moreover, this higher degree of rotting has resulted in the losses of "cellulose, etc." catching up those of furfuroids.

The most important aspect of these results is, however, the relation between the humification values and the actual losses of the different constituents. In this regard, the figures in the A columns of Table II must be studied. In both series it is obvious that there is no parallelism between the humification values and the losses of furfuroids: the highest humification values are given by clover hay, although the losses of furfuroids are relatively low, whilst in the cases of wheat straw and maize cobs, which give much lower humification values, the highest losses of furfuroids occurred. In the case of the losses of "cellulose, etc.", there is a general parallelism between these and the humification values when each series is considered separately, but when the results for the two series are compared it is seen that, both in the case of wheat straw and maize cobs, the losses of "cellulose, etc." in Series I are higher than in Series II, whereas the reverse is the case with regard to the humification values. Only in the case of the changes in "lignin values" is there a parallelism between the figures in the two series and the corresponding humification values.

These facts are more clearly brought out, if the ratios between the humification values and the losses of the various constituents (Columns A) are worked out. These ratios are shown in Table III.

Table III. *Relation between humification values and losses of different constituents.*

Material	"Cellulose, etc."	Furfuroids	"Lignin value"
Series I:			
Clover hay	5·0	2·6	2·0
Wheat straw	3·2	10·0	2·9
Maize cobs	3·9	29·0	2·7
Sawdust	10·0	9·4	4·3
Series II:			
Clover hay	10·1	1·9	2·2
Wheat straw	14·5	10·0	2·8
Maize cobs	19·6	12·2	2·7

The figures quoted under the heading of each constituent show the ratio of the loss of that constituent (see Columns A, Table II) to the humification value, for the material in question.

When expressed in this way, the ratios for "cellulose, etc." vary between 3·2 and 19·6, those for furfuroids between 1·9 and 29·0, whereas

those for "lignin value" vary only between 2·0 and 4·3, and with one exception are all between 2 and 3.

In these experiments, therefore, the formation of humic matter bears a much more constant relation to the change in "lignin value" as a result of rotting than it does to the changes in "cellulose, etc." or in furfuroids.

The results obtained in Series III, in which the purified preparations of the individual plant constituents were used, are shown in Table IV. In this series, cellulose was determined by the method of Charpentier as modified by Gray and Chalmers at Rothamsted(7), by solution in Schweitzer's reagent and precipitation with hydrochloric acid. Starch was determined by hydrolysis with dilute hydrochloric acid and titration with Fehling's solution.

Table IV. *Incubation of purified preparations of plant constituents under inoculated and sterile conditions.*

		Set *A*—inoculated		Set *B*—Sterile	
Substance	Nitrogen added as	Loss during rotting (%)	Humification value	Loss during incubation (%)	Humification value
Cellulose	Ammonium carbonate	61·4	Nil	3·4	Nil
Xylan	,,	100	Nil	Nil	Nil
Starch	,,	100	Nil	58	Nil
Lignin	,,	—*	9·6	—*	10·0
,,	Glycine	—*	8·4	—*	10·8
,,	Protein	—*	9·2	—	—
,,	No N	—	—	—*	7·6
Glucose	Ammonium carbonate	100	Nil	39	Nil
,,	Glycine	100	Nil	38	Nil
,,	Protein	100	Nil	—	—
,,	No N	—	—	48	Nil
Xylose	Ammonium carbonate	100	Nil	32	Nil
,,	Glycine	100	Nil	18	Nil
,,	Protein	100	Nil	—	—
,,	No N	—	—	Nil	Nil
Protein	—	88·2	Nil	3	Nil

* The "lignin values" after incubation were not determined owing to an accident. The greater part of the lignin originally present was, however, recovered in all cases.

No great accuracy is claimed for the values for losses in Set *B*, as it was difficult in some cases to discriminate between losses due to decomposition during the preliminary autoclaving and those occurring during the incubation. This uncertainty is, however, without any bearing on the most important aspect of the results, since both in the inoculated set in which, with the exception of lignin, all or the greater part of the substances originally present were destroyed in the course of the incubation, and in the sterile set in which an uncertain but usually minor part of the substances was destroyed, no humic matter whatever was

formed. With lignin, however, humification values indicating the production of humic matter corresponding to about 10 per cent. of the original lignin were obtained, both in the inoculated and in the sterile set.

Some experiments were also carried out with the decomposition of cellulose (filter paper) in liquid nutrient medium, inoculated with (*a*) a pure culture of *Spirochaeta cytophaga*, (*b*) soil extract, (*c*) *Sphagnum* extract, the nitrogen being present in the medium in each case in either of two forms, sodium nitrate or glycine. Although the cellulose in all cases suffered considerable decomposition, no humic matter was formed.

The results given in this paper are not considered in more detail here, since it is more convenient to discuss these results along with those given in the preceding and next parts of this series (Parts II and IV), in their bearings on the origin of humic matter. Part V of this series is devoted to this further discussion.

SUMMARY.

Experiments are described in which the decomposition of various plant materials and of purified preparations of plant constituents, during rotting under neutral aerobic conditions in the presence of soil organisms, has been studied in relation to the formation of humic matter.

The formation of humic matter was more closely related to the change in the lignin content than to the change in content of the other groups of plant constituents estimated.

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(*Received March 3rd*, 1930.)

THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS. VII.

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An examination of the fungicidal properties of various spray-fluids containing sulphur in polysulphide sulphur form has been continued upon the lines of experiments already described in this *Journal* (1, 2, 3, 4). The fungus employed for the spray trials was again the powdery mildew of the hop (*Sphaerotheca Humuli* (DC.) Burr.), and the methods used and precautions in technique observed were similar to those in previous work.

A. Sodium polysulphide solutions.

Experiments carried out in 1923 and recorded in Part IV of this series (4), although insufficient in number to ascertain the exact percentage of polysulphide sulphur required for complete fungicidal action, indicated that the probable figure was about 0·1 per cent. Further trials were therefore carried out with various preparations which, on analysis, yielded the following figures:

Percentage:	I	II	III	IV
Monosulphide sulphur	4·32	3·74	0·60*	1·30
Polysulphide sulphur	11·67	11·82	4·16	5·26
Thiosulphate sulphur	5·06	5·18	0·61	0·32
Sulphate sulphur	0·00	0·00	0·003	0·005

* Owing to the estimation of the monosulphide sulphur in dilute solution being unsatisfactory, the accuracy of this figure is doubtful.

Samples I and II were commercial products ("Amberene") while Samples III and IV were prepared in the laboratory by the saturation of sodium hydroxide solutions with hydrogen sulphide, the resultant solution being boiled under a reflux condenser with excess of sulphur. After cooling, the polysulphide solutions were filtered and stored under air-tight conditions.

The spray fluids were prepared by diluting the concentrated solution to double the strength at which it was intended to be used, an equal volume of the spreader solution at double strength then being added. This solution was sprayed without delay, and, when possible, a check was obtained by the analysis of samples taken at the time of spraying.

Exp. 10/28. 0·12 per cent. polysulphide sulphur (Sample I) and 1 per cent. soft soap.

Fifteen leaves were sprayed. The solution proved fungicidal.

Exp. 17/28. 0·11 per cent. polysulphide sulphur (Sample III) and 1 per cent. soft soap.

Ten leaves were sprayed. The solution proved fungicidal on all the leaves, while by the tenth day the patches on all the control (unsprayed) leaves at the same nodes were densely powdery. On four of the sprayed leaves the patches were effaced or semi-obliterated by the action of the spray.

Exp. 20/28. 0·10 per cent. polysulphide sulphur (Sample III) and 1 per cent. soft soap.

Ten leaves were sprayed. The solution was fungicidal, the patches on several of the leaves being semi-effaced by its action.

In view of the pronounced action of the spray-fluids in Exps. 17, 20/28, more dilute solutions were next employed.

Exp. 30/28. 0·05 per cent. polysulphide sulphur (Sample III) and 1 per cent. soft soap.

Eleven leaves were sprayed. All the patches were killed on the sprayed leaves, while those on the control (unsprayed) leaves at the same nodes remained powdery.

Exp. 37/28. 0·05 per cent. polysulphide sulphur (Sample III) and 1 per cent. soft soap.

Nine leaves were sprayed. All the patches were killed, while those on the control (unsprayed) leaves remained densely powdery.

In Part IV of this series(4) a number of experiments with a proprietary sodium polysulphide preparation ("Sulfluid") were recorded. The trials were too few to ascertain the exact percentage of polysulphide sulphur necessary for complete fungicidal action. Solutions containing 0·12 per cent. polysulphide sulphur, when applied with 1 per cent. soft soap, proved fungicidal, whereas fungicidal action was not complete when only 0·03 per cent. polysulphide sulphur was present. These results are in accordance with those of the above experiments. Under the conditions of the trials sodium polysulphide solutions containing 0·05 per cent. polysulphide sulphur are fungicidal if applied with 1 per cent. soft soap.

For certain experiments in which the fungicidal properties of sodium polysulphide solutions were compared with those of calcium and barium polysulphides, it was necessary to employ a freshly prepared gelatine solution as the spreader[1], since, with the latter materials, soap is in-

[1] Frequent experiments in which soft soap was used at strengths of 0·5 and 1·0 per cent. and gelatine at 0·5 per cent. failed to show indications of fungicidal action.

compatible. To eliminate variations due to biological and external conditions the sprays to be compared were, with one exception, applied simultaneously to opposite leaves at the same node.

Exp. 34/28. 0·10 per cent. polysulphide sulphur (Sample III) and 0·5 per cent. gelatine.

Twelve leaves were sprayed, those at the same nodes being sprayed with barium polysulphide (Exp. 33/28). In both cases the solutions proved almost or quite fungicidal, all the mildew patches usually being killed or, very rarely, a few weak, more or less clustered conidiophores developed from the youngest patches.

Exp. 30/29. 0·11 per cent. polysulphide sulphur (Sample II) and 0·5 per cent. gelatine.

Six leaves were sprayed. The solution proved fungicidal on all the leaves except one, on which scattered conidiophores developed.

Exp. 17/29. 0·11 per cent. polysulphide sulphur (Sample IV) and 0·5 per cent. gelatine.

Twelve leaves were sprayed, those at the same nodes being sprayed with calcium polysulphide (Exp. 18/29). In both cases the solutions proved just fungicidal; while all the patches were killed on the majority of the leaves, on a few leaves some scattered conidiophores were produced at the edge of a few patches.

Exp. 7/29. 0·10 per cent. polysulphide sulphur (Sample IV) and 0·5 per cent. gelatine.

Ten leaves were sprayed, those at the same nodes being sprayed with lime sulphur (Exp. 8/29). The solution proved non-fungicidal, although in some cases the patches were so affected that they re-grew only weak, more or less scattered, conidiophores.

When applied with 0·5 per cent. gelatine as the spreader, sodium polysulphide solutions are not quite fungicidal at a strength of 0·10 per cent. polysulphide sulphur, but are fungicidal at a concentration of 0·11 per cent. polysulphide sulphur. The results suggest that, in the presence of gelatine, sodium polysulphide solutions are not as effective as when soft soap is employed as the spreader. Strictly comparative trials to confirm this point were not undertaken but it is of interest theoretically in its analogy to the case of sulphur itself. It has been shown, in Part VI of this series(5) that the fungicidal action of both dusting sulphur and "colloidal" sulphur is inhibited by gelatine and saponin used as the spreader.

In the comparative trials reported above the fungicidal properties of sodium polysulphide solutions are compared with those of barium and calcium polysulphide solutions. In Exps. 34, 33/28 solutions of sodium and barium polysulphides, and in Exps. 17, 18/29 and 7, 8/29,

of sodium and calcium polysulphides, at similar strengths of polysulphide sulphur and spreader, are shown to have a similar action on the fungus.

B. Potassium polysulphide solutions.

In 1923 an examination was made of the fungicidal properties of a commercial liver of sulphur and the conclusion was reached that, at a concentration of 0·092 per cent. polysulphide sulphur, the solution was completely fungicidal when applied with 0·5 per cent. soft soap solution.

In the experiments described below, two samples of potassium polysulphide solution were prepared in the laboratory by a method similar to that used for the preparation of the sodium polysulphide solutions III and IV. Analyses of the two solutions gave the following figures:

Percentage:	V	VI
Monosulphide sulphur	1·07	0·76
Polysulphide sulphur	4·11	3·09
Thiosulphate sulphur	1·04	0·09
Sulphate sulphur	0·00	0·01

The following three experiments were carried out with Sample V:

Exp. 21/28. 0·10 per cent. polysulphide sulphur and 1 per cent. soft soap.

Seven leaves were sprayed. The solution proved fungicidal; some of the patches were effaced or semi-obliterated by the action of the spray. All the patches on the control (unsprayed) leaves at the same nodes remained powdery.

Exp. 29/28. 0·05 per cent. polysulphide sulphur and 1 per cent. soft soap.

Twelve leaves were sprayed. The solution proved fungicidal. All the patches on the control (unsprayed) leaves at the same nodes remained powdery.

Exp. 38/28. 0·05 per cent. polysulphide sulphur and 1 per cent. soft soap.

Ten leaves were sprayed. Exactly the same results were obtained on the sprayed and unsprayed leaves as in Exp. 29/28.

When applied with 1·0 per cent. soft soap, potassium polysulphide solutions are fungicidal at a concentration of 0·05 per cent. polysulphide sulphur. This figure is not in complete agreement with the 1923 result when a solution of 0·066 per cent. polysulphide sulphur and 1 per cent. soft soap was found to be almost but not quite fungicidal. The main point of interest, however, is the similarity of the above results with those obtained with sodium polysulphide solutions (Exps. 20, 30 and 37/28).

The similarity between the behaviour of sodium and potassium polysulphide solutions at the same polysulphide sulphur content is likewise shown when gelatine is added as the spreader.

Exp. 26/29. 0·11 per cent. polysulphide sulphur (Sample VI) and 0·5 per cent. gelatine.

Nine leaves were sprayed. The solution proved fungicidal on seven leaves; on the remaining two leaves, scattered or clustered conidiophores developed on a few of the patches. All the patches on the control (unsprayed) leaves remained powdery.

Exp. 10/29. 0·10 per cent. polysulphide sulphur (Sample VI) and 0·5 per cent. gelatine.

Six leaves were sprayed, those at the same nodes being sprayed with calcium polysulphide solution (Exp. 9/29). In both cases the solutions appeared to be not quite fungicidal, the patches on several of the leaves producing a few conidiophores.

The results again suggest that the nature of the spreader may affect the fungicidal activity of polysulphide sulphur. In the comparative trial with calcium polysulphide solution, the effect of the sprays is in agreement with the hypothesis that polysulphide sulphur gives a measure of fungicidal efficiency independent of the base present.

C. CALCIUM POLYSULPHIDE SOLUTIONS.

Solutions containing calcium polysulphides were prepared in the laboratory not only by the method used in the case of sodium and potassium polysulphide solutions (Sample VIII) but also by boiling suspensions of sulphur in milk of lime (Samples IX and X). Analyses of the various solutions together with that of a commercial lime sulphur (Sample VII) are included in the following table:

Percentage:	VII	VIII	IX	X
Monosulphide sulphur	5·04	1·46	2·00	1·45
Polysulphide sulphur	19·20	6·53	8·76	5·67
Thiosulphate sulphur	0·62	0·15	2·37	1·87
Sulphate sulphur	0·00	0·00	0·00	0·00

It will be noticed that the four solutions fall into two groups, Samples VII and VIII in which the proportion of thiosulphate sulphur to total sulphur is small, and Samples IX and X in which the relative amount of thiosulphate sulphur is high.[1]

In previous experiments with calcium polysulphide solutions the

[1] It may be mentioned that attempts to prepare solutions in which the proportions of monosulphide to polysulphide sulphur showed similar variations failed. A similar failure was attributed by Auld (6) to the precipitation of excess lime in the form of Herschell's crystals.

spreader employed was a solution of calcium caseinate (lime-casein). To bring the trials recorded below into line with trials of other polysulphide solutions, freshly-prepared gelatine solutions were used as the spreader.

Trials, including two under strictly comparable conditions, gave the following results:

Exp. 25/29. 0·11 per cent. polysulphide sulphur (Sample VIII) and 0·5 per cent. gelatine.

Ten leaves were sprayed. The solution proved almost or completely fungicidal; while all the patches were killed on most of the leaves, on the other leaves some of the younger patches re-developed a few conidiophores. The patches on all the control (unsprayed) leaves remained densely powdery. The behaviour of the solution corresponded exactly with that of the potassium polysulphide in Exp. 26/29 (see above).

Exp. 27/29. 0·11 per cent. polysulphide sulphur (Sample IX) and 0·5 per cent. gelatine.

Ten leaves were sprayed. The solution proved fungicidal on five leaves; on the remaining leaves the older patches were killed but a few conidiophores developed from the younger patches. All the patches on the control (unsprayed) leaves remained powdery.

Exp. 31/29. 0·11 per cent. polysulphide sulphur (Sample X) and 0·5 per cent. gelatine.

Seven leaves were sprayed. The solution proved fungicidal for all the patches on five leaves, while on the remaining two leaves a few clustered conidiophores developed from younger patches. The patches on all the control (unsprayed) leaves remained powdery.

Exp. 51/29. 0·11 per cent. polysulphide sulphur (Sample VII) and 0·5 per cent. gelatine.

Exp. 52/29. 0·11 per cent. polysulphide sulphur (Sample X) and 0·5 per cent. gelatine.

Nine leaves at the same nodes were sprayed. In both cases the solutions proved fungicidal.

Exp. 61/29. 0·10 per cent. polysulphide sulphur (Sample IX) and 0·5 per cent. gelatine.

Exp. 62/29. 0·10 per cent. polysulphide sulphur (Sample VIII) and 0·5 per cent. gelatine.

Eighteen leaves at the same nodes were sprayed. In both cases the solutions proved fungicidal.

The results indicate that at a concentration of 0·10 to 0·11 per cent. polysulphide sulphur the various calcium polysulphide solutions (when applied with 0·5 per cent. gelatine) are fungicidal. The behaviour of the

various sprays, despite their different content of total sulphur and of thiosulphate sulphur, is dependent upon the content of polysulphide sulphur.

In the following experiments the behaviour of calcium polysulphide solutions with the same spreader was compared with that of sodium, potassium and barium polysulphides under similar biological and external conditions.

Exp. 8/29. 0·10 per cent. polysulphide sulphur (Sample VII) and 0·5 per cent. gelatine.

Ten leaves were sprayed, those at the same nodes being sprayed with sodium polysulphide (Exp. 7/29). The solution proved to be not quite fungicidal; some patches were killed but others re-formed scattered or clustered conidiophores.

Exp. 18/29. 0·11 per cent. polysulphide sulphur (Sample VII) and 0·5 per cent. gelatine.

Twelve leaves were sprayed, those at the same nodes being sprayed with sodium polysulphide (Exp. 17/29). In both cases the solutions proved to be just fungicidal.

Exp. 12/29. 0·09 per cent. polysulphide sulphur (Sample VIII) and 0·5 per cent. gelatine.

Ten leaves were sprayed, those at the same nodes being sprayed with barium polysulphide (Exp. 11/29). In both cases the solutions proved to be not quite fungicidal; while some were killed, most of the patches re-formed clustered conidiophores.

Exp. 9/29. 0·10 per cent. polysulphide sulphur (Sample IX) and 0·5 per cent. gelatine.

Six leaves were sprayed, those at the same nodes being treated with potassium polysulphide (Exp. 10/29). The material proved to be rather poor, several of the leaves yellowing before the end of the experiment; so far as could be judged both solutions were just below fungicidal strength.

The experiments confirm the view that the response of the fungus towards solutions of calcium, sodium, potassium and barium polysulphides is determined by polysulphide sulphur content.

D. Barium polysulphide solutions.

The direct estimation of polysulphide sulphur present in solutions of barium polysulphide by the methods used in previous work(7) is frustrated by the relative insolubility of the barium thiosulphate formed. This difficulty may, however, be overcome by the preliminary precipitation of the barium as sulphate by the addition of excess sodium sulphate. Ammoniacal zinc chloride solution is then added, and the precipitated sulphur determined by the estimation of the thiosulphate

formed when the mixture is heated on a boiling water-bath with excess of sodium sulphite.

Two solutions containing barium polysulphides were prepared in the laboratory by the saturation of barium hydroxide solution with hydrogen sulphide. The resultant solution was boiled under a reflux condenser with excess of elementary sulphur and the cooled solution filtered and stored in air-tight bottles. Analysis gave the following figures:

Percentage:	XI	XII
Monosulphide sulphur	0·72	0·74
Polysulphide sulphur	2·34	3·36
Thiosulphate sulphur	0·12	0·05
Sulphate sulphur	Nil	Nil

As in the case of the calcium polysulphide solutions, a freshly prepared 0·5 per cent. solution of gelatine was used as the spreader in the following spray trials:

Exp. 15/28. 0·11 per cent. polysulphide sulphur (Sample XI).

Eight leaves were sprayed, those at the same nodes being sprayed with 0·5 per cent. gelatine. The barium polysulphide solution proved non-fungicidal for all the patches on five of the leaves; on the remaining leaves the oldest patches were killed, but conidiophores developed from the younger. The gelatine solution proved non-fungicidal, all the patches re-forming densely clustered conidiophores by the third day after spraying.

Exp. 24/29. 0·12 per cent. polysulphide sulphur (Sample XII).

Nine leaves were sprayed. The solution proved fungicidal for all the patches on eight leaves; on the ninth leaf conidiophores developed from several of the patches. All the patches on the control (unsprayed) leaves remained densely powdery.

Exp. 11/29. 0·11 per cent. polysulphide sulphur (Sample XII).

Ten leaves were sprayed, those at the same nodes being sprayed with calcium polysulphide (Exp. 12/29). The solution proved to be not quite fungicidal; while some were killed, most of the patches re-formed clustered conidiophores.

Exp. 33/28. 0·10 per cent. polysulphide sulphur (Sample XI).

Twelve leaves were sprayed, those at the same nodes being sprayed with sodium polysulphide (Exp. 34/28). The solution proved to be almost or quite fungicidal, all the patches usually being killed or, very rarely, a few weak, more or less clustered conidiophores developed from the youngest patches.

The results indicate that, when applied with 0·5 per cent. gelatine as the spreader, barium polysulphide solutions are fungicidal at a concentration of approximately 0·11 per cent. polysulphide sulphur. Further they show that, under similar conditions, the behaviour as fungicides of barium polysulphide solutions is similar to that of sodium, potassium and calcium polysulphide solutions at the same concentration of polysulphide sulphur.

SUMMARY.

1. The fungicidal properties of solutions containing sulphur in polysulphide form, viz. sodium, potassium, calcium and barium polysulphides, were tested by application to the conidial stage of *Sphaerotheca Humuli* growing upon young hop leaves in the greenhouse.

2. Four samples of sodium polysulphide solutions, two prepared in the laboratory and two commercial products, were examined. When applied with 1 per cent. soft soap as the spreader, solutions containing 0·5 per cent. polysulphide sulphur proved fungicidal. With 0·5 per cent. gelatine as the spreader, fungicidal strength did not begin to be attained until the concentration of polysulphide sulphur reached 0·11 per cent. The results suggest that the nature of the spreader may influence the fungicidal properties of polysulphide sulphur.

3. Two samples of potassium polysulphide solutions prepared in the laboratory had an action similar to that of sodium polysulphide solutions when applied at the same concentration of spreader and polysulphide sulphur.

4. Four samples of calcium polysulphide solutions including one commercial lime sulphur proved almost or quite fungicidal at concentrations of 0·11 per cent. polysulphide sulphur when applied with 0·5 per cent. gelatine.

5. Two laboratory-prepared barium polysulphide solutions proved almost or quite fungicidal at a content of 0·11 per cent. polysulphide sulphur when applied with 0·5 per cent. gelatine.

6. Solutions of calcium polysulphide which differed widely in the relative amounts of total and thiosulphate sulphur behaved as fungicides in accordance with the polysulphide sulphur content only.

7. The fungicidal efficiency of the polysulphide sulphur is shown to be independent of the base with which it is combined.

8. The results confirm the view that the polysulphide sulphur content is a measure of the fungicidal power of spray-fluids of the polysulphide class.

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(*Received March 8th*, 1930.)



VOLUME XX OCTOBER, 1930 PART 4

THE REPRESSIVE EFFECT OF LIME AND MAGNESIA UPON SOIL AND SUBSOIL POTASH.

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STANDARD English and American texts teach that liming effects a liberation of soil potash through ionic interchange. As examples we have the statement of Hall(8): "The action of lime upon potash compounds in the soil is equally marked: as the soil water carries down the dissolved calcium bicarbonate it attacks the zeolitic double silicates in the clay and some of their soluble bases, potash among them, change place with the lime and come into solution." Similar statements, "One of the most important effects of calcium compounds is the conversion of insoluble into soluble forms of potassium..." by Van Slyke(25) and "but it also has some power to increase the solubility of phosphorus and potassium..." by Hopkins(9), have been accepted as authoritative.

For a number of years the several effects produced by different calcic, magnesic and dolomitic materials in the soil have been studied at the University of Tennessee Agricultural Experiment Station. This paper deals with the influence of lime-magnesia upon potash solubility and is based upon the findings secured by means of five lysimeter studies with fallow soils over a 15-year period.

EXPERIMENTAL.

The rates of liming have extended from 250 lb. to 200,000 lb. of CaO equivalence per 2,000,000 lb. of soil. In the 12-year and 15-year studies, and also in the three 4-year experiments, the water-soluble potash content of the rainfall percolates from each of the 147 tanks has been determined annually. The chlor-platinate colorimetric method (24) was used, especial care being taken to preclude interference from ammonia contamination.

THE 12-YEAR LIME-MAGNESIA-SULPHUR EXPERIMENT.

In this experiment an 8 inch depth of a brown loam was used without subsoil. The soil was acid and had never been limed. The total potassium content per 2,000,000 lb. of soil was 15,060 lb. of which 4860 lb. was soluble in 1·115 HCl and 128 lb. in 0·2*N* HNO_3. No potash was added.

One group of seven lysimeters was used for controls, and three groups of five tanks each were used for CaO and MgO additions with the three 1000 lb. sulphur-equivalent supplements, ferrous sulphate, pyrites and flowers of sulphur. The immediate acidity of the ferrous sulphate addition and the potential acidity of the oxidisable pyrites and sulphur additions required 1750 lb. of CaO for neutralisation. A 3750 lb. addition of CaO, and its MgO equivalent, were therefore included, along with controls of 2000 lb. of CaO and its MgO equivalent. The effect of excessive amounts of CaO and MgO along with the sulphur carriers was also studied, 32 tons of CaO or its equivalent of MgO being used. In the group of controls, 2000 lb. CaO equivalents of 100-mesh limestone and 100-mesh dolomite were also included. All treatments were mixed throughout the full depth of soil.

The total losses of potassium carried from the control group by an average rainfall of 51 inches are given in Table I. In every instance the potassium loss was less from the limed than from the unlimed soil.

Table I. *The effect of liming on the outgo of potassium from a brown loam soil over a period of* 12 *years.*

Total leached—lb. K per 2,000,000 lb. of soil.

	100-mesh limestone, 1 ton CaO	100-mesh dolomite, 1 ton CaO	Burnt lime		Burnt magnesia	
Control	=O=	=O=	1 ton	$1\frac{3}{4}$ tons	1 ton =O=	$1\frac{3}{4}$ tons =O=
101	95	79	87	87	73	83

Table II. *The effect of liming, with and without, supplementary sulphur materials*, on the outgo of K from a brown loam soil over a period of* 12 *years.*

Total leached—lb. K per 2,000,000 lb. of soil†.

CaO, MgO additions		Ferrous sulphate	Pyrites additions	Flowers of sulphur additions
Form	Amount			
—	—	99	102	105
CaO	$1\frac{3}{4}$ tons‡	74	71	77
MgO	$1\frac{3}{4}$ tons=O=	79	67	74
CaO	32 tons	77	70	75
MgO	32 tons =O=	69	63	59

* A constant of 1000 lb. of sulphur.
† The outgo from the untreated soils (Table I) was 101 lb.
‡ $\frac{7}{8}$ ton required to neutralise the immediate or potential acidity of sulphur materials.

In Table II are summarised the 12-year leachings from the tanks that provided an amount of soluble neutral calcium and magnesium salts equivalent to 4250 lb. of $CaSO_4$, as supplementary to the bicar-

bonates of calcium and magnesium that were derived from the CaO and MgO additions. In the case of the ferrous sulphate, this $CaSO_4$ equivalence of neutral salts was developed immediately. On the other hand, there was a progressive increment of sulphates from the oxidation of the pyrites and sulphur additions. All of the sulphate radical that was added as ferrous sulphate and all that was engendered from the pyrites and the sulphur came from the soil as $CaSO_4$ and $MgSO_4$. This was true for both the sulphur-carrier controls, without CaO or MgO, and the several sulphur additions that were incorporated along with the two caustic materials. The ferrous sulphate reacted with the oxides, carbonates, or silicates, of calcium and magnesium, to produce calcium and magnesium sulphates in the leachings, without any indication of an accompanying outgo of iron, as indicated by the equations:

$$CaO + FeSO_4 + H_2O \rightarrow CaSO_4 + Fe(OH)_2,$$

$$CaCO_3 + FeSO_4 + 2H_2O \rightarrow CaSO_4 + Fe(OH)_2 + H_2CO_3,$$

$$CaSiO_3 + FeSO_4 + H_2O \rightarrow CaSO_4 + Fe(OH)_2 + SiO_2,$$

and $$2Fe(OH)_2 + O = Fe_2O_3 + 2H_2O.$$

The data of Table II demonstrate that, as measured by totals, there was no indication of potassium replacement by either the heavy initial increment of $CaSO_4$-$MgSO_4$ derived from the ferrous sulphate, or the progressive increments engendered from the other two forms of sulphur. In the initial periods, however, there was some indication(20) of a *slight* liberation of potassium by the addition of the sulphur materials, *without lime or magnesia.*

It is apparent, however, that the added CaO and MgO exerted a repressive, or buffering, effect that prevented the hydrolysis of those natural soil components that contained potassium. This protective effect was exerted to the greatest extent by the heavy magnesia additions—those that gave the greatest concentrations of bicarbonates in the percolates.

The 15-year experiment with seven liming materials.

In this experiment the brown loam used was somewhat "heavier" than the phase used in the 12-year experiment. Of its total K content of 20,080 lb. per 2,000,000 lb. of soil, 4880 lb. was soluble in 1·115 HCl and 194 lb. was found to be soluble in 0·2*N* HNO_3. No potash addition was made, and the seven liming materials were incorporated throughout the full depth of soil.

Forty-four tanks were used, twenty-two with surface soil only and twenty-two in which the surface soil was underlaid with 1 foot of red clay subsoil. This permitted both the direct determination of the effect of the additions on the surface soil and the tendency of the subsoil stratum to retain the bases that were leached from the soil.

Table III. *The effects of seven liming materials at three excessive rates, on the outgo of potassium from soil and soil-subsoil horizons over a 15-year period*.*

Total leached—lb. K per 2,000,000 lb. of soil.

	Burnt lime		Burnt magnesia		Ppt. $CaCO_3$		Ppt. $MgCO_3$		100-mesh limestone		100-mesh dolomite		100-mesh magnesite	
CaO equiv. tons	Soil only	Soil and subsoil	Soil only	Soil and subsoil	Soil only	Soil and subsoil	Soil only	Soil and subsoil	Soil only	Soil and subsoil	Soil only	Soil and subsoil	Soil only	Soil and subsoil
8	112	121	103	124	126	137	116	124	114	134	102	136	114	128
32	161	154	101	124	135	144	93	116	119	134	105	132	99	128
100	392	111	133	110	122	112	91	92	117	141	99	140	118	149
Av.	222 (137)†	129	112	119	128	131	100	111	117	136	102	136	110	135
Av. outgo from subsoil	—	—	—	7	—	3	—	11	—	19	—	34	—	25

Controls: Soil only, 192. Soil and subsoil, 220.

* 8 inch depth of soil and 12 inch depth of red clay subsoil.
† Average of 8 and 32 tons only.

Surface soil only. The data of Table III show that the total outgo of 192 lb. from the untreated surface soil was practically identical with the amount of replaceable potassium found in the soil at the beginning of the experiment. This outgo from the unlimed soil was neither equalled, nor approached, by that from any of the six materials, MgO, $3MgCO_3$, $Mg(OH)_2$, limestone, dolomite, and magnesite, at any of the three CaO equivalent rates of 8, 32, or 100 tons. The average total outgo of potassium induced by these eighteen treatments was only 112 lb., as against 192 lb. for the untreated soil. Neither the 8 ton nor the 32 ton burnt-lime treatment gave so large a yield as the control. The single exception where liming produced an increase in potassium outgo was found where the enormous addition of 100 tons of unslaked burnt lime (CaO) had been made. In this case a very high temperature was produced by the slaking process at the time of incorporation. For nearly 3 years the percolates from this treatment contained $Ca(OH)_2$ and were devoid of bicarbonates (16). It was only during this 3-year period, and more especially during a single year of excessive rainfall, that potassium liberation was induced. To a lesser extent, as to amount and time, there was a similar tendency in the case of the 32 ton addition. When the

calcium hydroxide had all reverted to the carbonate, there was no further indication of potassium liberation during any of the twelve subsequent annual periods.

Surface soil underlaid by subsoil. The losses of potassium from the untreated soil and subsoil amounted to 220 lb. as against 192 lb. from the surface soil alone. The outgo from the 1 foot depth of clay subsoil was, therefore, only 14·6 per cent. of that found for the 8 inch depth of surface soil, although the percentage content of potassium in the clay was 1·44 times that found for the surface soil. This 28 lb. difference between soil and soil-subsoil outgo was diminished in every instance when increments of calcium and magnesium came to the subsoil from the 8 ton treatments. The average of the seven 8 ton additions gave a surface soil outgo of 112 lb., as against that of 129 lb. from the corresponding soil-subsoil tanks. Thus the influx of the Ca-Mg-impregnated surface soil percolates caused either an absorption of potassium by the subsoil or a diminution in its own outgo, or both.

A similar result was obtained—an increment from the subsoil—for all of the 32 ton additions, with the exception of burnt lime. In the case of the 100 ton additions, this exception applied to both the oxide and the carbonate, whereas the three natural products, limestone, dolomite, and magnesite, behaved as did their 8 ton and 32 ton additions. In the case of the 100 ton additions of burnt lime the enhanced outgo of potassium from the surface soil alone was 200 lb., whereas the difference between the unlimed soil-subsoil control and the limed soil-subsoil was only 109 lb. It is, therefore, evident that the absorptive, or fixing, capacity of the subsoil was materially enhanced by the influx of calcium from the heavily limed surface soil.

Four-year experiment with "light," "heavy" and crystalline magnesic compounds.

In this experiment, so far as we are aware, the "light" and "heavy" forms of oxide and carbonate were used in comparison with crystalline magnesium carbonate, $MgCO_3.3H_2O$, for the first time. The several materials were mixed throughout the full depth of soil immediately before placement in the tanks. The soil was similar to the cultivated soil of Table III. The present soil, however, had been in grass for a number of years. The overlying strip of turf was removed and the adjacent lower layer was used.

The results of Table IV show that each of the eight magnesic additions caused a decrease in the outgo of potassium. The losses of potassium

during the initial year were heavier than those for the subsequent years, during which there was a sustained relationship between the normal outgo from the untreated soil and the repressed outgo induced by each magnesic addition.

Table IV. *The effects of five magnesic materials on the outgo of potassium from a brown loam soil over a 4-year period.*

Magnesic additions		lb. K_2O leached per 2,000,000 lb. of soil				
Form	CaO ≒ (tons)	1st year	2nd year	3rd year	4th year	4-year total
Control	—	70	74	42	52	238
"Light" magnesia	8	51	44	37	38	170
"Heavy" magnesia	8	56	42	31	42	171
"Light" magnesia carbonate	8	39	44	28	39	150
"Heavy" magnesia carbonate	8	59	40	32	41	172
Crystalline magnesia carbonate	8	68	40	27	40	175
"Light" magnesia	32	68	30	26	34	158
"Light" magnesia carbonate	32	57	32	19	28	136
Crystalline magnesia carbonate	32	51	37	16	34	138

Potassium outgo induced by surface zone and sub-surface zone incorporations of liming materials.

In each of the foregoing experiments the liming materials were incorporated with the entire body of the soil. In practice, however, they are usually disced into the soil, so that the actual contact between soil and liming materials is, for a time, mainly in the upper zone. Until the soil is turned the dissolved calcium and magnesium pass from this zone of contact into the lower zone before passing into the subsoil. This aspect of the problem was considered in an experiment wherein incorporations of $Ca(OH)_2$ were made (*a*) in the upper half only, and (*b*) in the lower half only, without subsoil, and at the rates of 250, 500, 1000 and 2000 lb. of CaO. Burnt dolomite, separately calcined CaO + MgO, and limestone and dolomite separates of five degrees of fineness and a constant equivalence of 2000 lb. of CaO, were also made in the same manner. The results from the eighteen pairs of tanks are given in Table V.

Surface zone additions. For the four $Ca(OH)_2$ additions and those of limestone and dolomite it may be said that the gravitation of neutral salts of calcium and magnesium and a minimum movement of bicarbonates resulted in a small replacement of potassium by calcium in the lower acid zone. The actual replacements were probably greater than the net results, in that the percolates had to liberate enough potassium from the lower zone to offset the repressive effect exerted in the upper zone of direct soil-lime contact and still show an enhancement in the

outgo from the full depth. In the case of the burnt dolomite and the CaO-MgO additions, however, there was no such indication. The greater movement of the more soluble magnesium bicarbonate was apparently sufficient to impart some repressive effect in the lower zone, as well as in the zone of contact.

Table V. *Potassium outgo induced by additions of hydrated lime, burnt dolomite, and limestone and dolomite of different degrees of fineness to the upper and lower zones of a brown loam that had been in grass for over* 30 *years.*

Materials added		lb. K leached per 2,000,000 lb. of soil									
		From upper-zone additions					From lower-zone additions				
Form and mesh	CaO equiv.	1st year	2nd year	3rd year	4th year	4-year total	1st year	2nd year	3rd year	4th year	4-year total
Average controls	—	23·8	15·2	8·1	6·1	53·2	23·8	15·2	8·1	6·1	53·2
$Ca(OH)_2$	250	23·8	15·6	10·2	5·5	55·1	20·1	17·6	8·5	4·9	51·1
$Ca(OH)_2$	500	30·3	18·7	6·8	6·6	62·4	24·9	14·7	6·9	4·3	50·8
$Ca(OH)_2$	1000	30·2	15·4	9·3	7·6	62·5	20·5	12·0	5·5	4·3	42·3
$Ca(OH)_2$	2000	25·3	16·7	10·7	6·2	58·9	20·7	13·5	5·6	4·5	44·3
Burnt dolomite	2000	22·2	14·5	5·6	5·7	48·0	19·8	12·3	7·1	5·2	44·4
CaO-MgO mixture	2000	21·8	14·2	6·5	3·8	46·3	17·8	10·0	6·0	5·2	39·0
Limestone 10– 20	2000	26·5	18·6	7·9	5·8	58·8	26·6	10·7	4·2	4·2	45·7
Limestone 20– 40	2000	25·3	18·5	7·7	7·6	59·1	23·7	12·6	9·0	5·0	50·3
Limestone 40– 80	2000	24·4	17·1	11·9	6·0	59·4	22·6	13·0	6·9	4·6	47·1
Limestone 80–200	2000	22·6	20·6	11·5	5·4	60·1	21·6	10·3	8·1	4·5	44·5
Composite*	2000	18·1	27·0	5·1	3·9	54·1	20·1	9·9	6·1	3·9	40·0
Dolomite 10– 20	2000	24·0	14·6	10·6	5·2	54·4	22·4	12·5	7·7	5·0	47·6
Dolomite 20– 40	2000	22·1	17·5	9·3	6·2	55·1	22·3	10·3	7·4	6·0	46·0
Dolomite 40– 80	2000	24·5	15·7	7·4	5·5	53·1	13·8	6·8	7·0	4·5	32·1
Dolomite 80–200	2000	22·0	19·8	7·3	6·9	56·0	13·4	11·5	12·0	5·7	42·6
Composite*	2000	25·9	16·4	10·2	6·7	59·2	21·7	15·1	9·3	5·8	51·9

* Equal parts of 10–20, 20–40, 40–80 and 80–200 mesh separates.

Subsurface zone incorporations. In the group of lower zone incorporations, wherein all of the percolates from the upper zone were uninfluenced by any addition of lime, there was a lessened outgo of potassium in every instance where the 2000 lb. incorporations were made. The effect produced by the 250 lb. and 500 lb. additions, if any, was small.

Repressive effect of lime on the solubility of added potash.

In each of the four experiments that have been discussed the repressive action of lime and magnesia was exerted solely on the natural supplies of potassium in the soil, except for the small increment from rainwater (18). In a more recent experiment, potash additions of 70 lb. and 280 lb. of water-soluble K_2O were made through the incorporation of clover hay at the rates of 2 and 8 tons. Table VI gives a summary of a recent detailed contribution (19) dealing with the conservation of

the clover-derived potash, when additions of $Ca(OH)_2$, 40–50-mesh and 100-mesh limestone, and dolomite, were also made.

Table VI. *The effects of liming materials* on the 4-year outgo of potassium from a brown loam soil that had received additions of red clover hay.*

K_2O leached—lb. per 2,000,000 lb. of soil by 219 inches rainfall.

From soil alone	From soil and three liming materials†	From soil and 2 tons clover	From soil, 2 tons clover and three liming materials†	From soil and 8 tons clover	From soil, 8 tons clover and three liming materials†
155	120	194	144	292	270

* Constant CaO equivalence of 2000 lb. per 2,000,000 lb. of soil.

† Average of ten treatments—two of $Ca(OH)_2$ and two each of 40–50-mesh and 100-mesh separates of both high calcic and dolomitic limestones.

Again the liming materially reduced the outgo of native potassium. It also produced a distinct repressive effect upon the outgo of the added potash. This was especially marked in the case of the 70 lb. addition of K_2O that was supplied by the 2 tons of clover hay. In the case of the 8 ton addition, the 280 lb. K_2O increment was supplemented by a CaO-MgO content equivalent to 976 lb. of $CaCO_3$. Apparently this was sufficient to exert in some degree the retardation that was induced by liming, for the 8 ton clover control showed an abnormal retentive power for potash. It is also shown that greatly enhanced quantities of CO_2 generated within the soil did not overcome the repressive effect of the liming materials. The effect of liming upon the retention of added potash salts—nitrate, chloride, and sulphate—is now being studied by means of the lysimeter.

General discussion.

In earlier progress reports comprehensive bibliographies of the subject were given (17, 20, 21). For the present discussion it is sufficient to say that the teaching as to the supposed liberation of potash by liming materials was arrived at by deductions from laboratory experiments. In those experiments, soils were suspended in solutions of neutral alkaline-earth salts to obtain filtrates, the analysis of which showed that an interchange between calcium and potassium ions had taken place. Analyses for total residual potash on the limed plots of the Pennsylvania Station (15) and on soils from cylinders at the New Jersey Station (11) gave support to this assumption. Plant-ash (1, 2, 6, 10, 14, 26) analyses generally showed a decreased potash content in those crops that had been grown on limed soils, and it was assumed that the decrease was

due to a diminished supply of available soil potash as a result of calcium replacement, rather than a decreased solubility induced by the liming.

The alternative interpretation, that of a decrease in potash solubility induced by liming, was advanced by Gaither(7) in 1910, in referring to unpublished data obtained by the analysis of wheat that had been grown upon soil limed at the rate of 2000 lb. of CaO per acre. He stated that the data "seem to give good grounds for the conclusion that the addition of lime to those soils decreases the potassium that may be assimilated by the crop...." Several other workers interpreted their findings as at least casting doubt upon the assumption that soil potash is replaced by liming materials. Lyon and Bizzell(12, 13) failed to obtain any enhancement in the potassium content of the percolates from a 3000 lb. CaO addition to the surface soil of a 4 foot column of soil and subsoil. But the addition of potassium sulphate without lime likewise failed to show any enhancement in the potassium content of the percolates. They therefore concluded that lime either failed to exert a liberation, or that any actual liberation was masked by the absorptive capacity of the subsoil—"If there is any liberation of potassium by lime, the potassium must have been absorbed by the lower layers of soil and thus did not appear in the leachings"(12). The present finding demonstrates the value of a parallel series of shallow tanks without subsoil and deeper tanks with both soil and subsoil in order to determine definitely the exact horizon in which the transformations registered by the nutrient content of the percolates take place.

Each of the five Tennessee studies has demonstrated that, when increases in hydrolysable calcic and magnesic compounds ensue as a result of the fixation of added lime and magnesia, there follows a depression in the hydrolysis of potassic compounds. The solvent action that is exerted by carbonated soil-water on native supplies of potash decreases as the quantities of bicarbonates of calcium and magnesium increase. From the results of Plummer(22) and those of Briggs and Breazeale(4) it is certain that bicarbonate-impregnated waters have no solvent action upon mineral potassic compounds, whereas the present contribution demonstrates that the same is true also for the colloid complex. Moreover, the greater the concentrations of bicarbonates, the less will be the hydrolytic breakdown of the potash-bearing colloid complex. This was true for each form of high calcic, high magnesic, and dolomitic material in economic amounts, and also for excessive additions, with a single exception—the 100 ton CaO addition. The *protective effect* that the bicarbonate-impregnated percolates exerted upon the 1 foot

depth of clay subsoil is the *opposite* of that postulated by Hall(8); "as the soil water carries down the dissolved calcium bicarbonate it attacks the zeolitic double silicates in the clay and some of their soluble bases, potash among them, change place with the lime and come into solution."

It is certain that lime additions do not drive potash out of the soil complex and into the free soil water. The Tennessee Station viewpoint is that added lime drives potash back into the soil. This increase in the proportions of solid-phase potash and the corollary decrease in solute potash is naturally reflected in the decreased quantity of potassium found for plants grown on limed soils(1, 2, 10, 14). The thought that the presence of calcium bicarbonate in the soil water is responsible for a protective, or buffering, effect upon the potassic compounds has been expressed also by Ramann and Junk(23).

A recent observation was made concerning a potash deficiency that was registered by a crop of barley grown on land that had been limed in preparation for a lucerne crop which was not sown(3). Buie *et al.*(5) found that unnecessary repetitions of liming gave every indication of potash deficiency. It is quite likely that the addition of lime may depress the solubility of both native and added supplies of potassium to the point of creating a potash deficiency for certain crops. It does not follow, however, that this will hold for all crops. Plants such as clover are undoubtedly benefited by the correction of soil acidity and calcium deficiency, and by increased bacterial activities. They thus develop a great capacity to forage for their potassium requirements.

It seems definitely determined, however, that under humid conditions it is fallacious to expect that the addition of lime will accomplish any direct chemical liberation of soil potash in rock-derived soils.

Summary.

It is pointed out that authoritative texts maintain that liming results in an ionic exchange between added calcium and native potassium.

Data from five lysimeter studies conducted over a 15-year period at the University of Tennessee Agricultural Experiment Station are given to show the effect of calcic and magnesic compounds on the solubility of potassium in soil and subsoil.

A 12-year study shows that (*a*) economic additions of CaO, MgO, limestone and dolomite depressed the solubility of native supplies of potassium when the incorporations are made throughout the soil; (*b*) 1 ton and 32 ton additions of CaO and MgO produced the same repressive effect, even when supplemented by excessive immediate

quantities of sulphates of calcium and magnesium from ferrous sulphate and progressive increments of the same salts from pyrite and sulphur.

A 15-year study with excessive quantities of seven forms of lime and magnesia showed (*a*) a decided decrease in the potash solubility in the surface soil, and (*b*) a marked decrease in the solubility of potassium in the subsoil, as a result of the influx of bicarbonate-impregnated percolates. The single exception came from the 100 ton addition of CaO, and then only during the persistence of $Ca(OH)_2$.

A 4-year study with "light" and "heavy" forms of oxide and carbonate of magnesium and crystalline magnesium carbonate ($MgCO_3.3H_2O$) showed the same consistent depression in potash solubility.

Surface zone incorporations of hydrated lime, limestone, and dolomite, produced neutral-salt-impregnated percolates that gave some indication of potassium liberation in an underlying unlimed zone of surface soil. Sub-surface zone incorporations produced the same repressive effect that had been obtained in the full depth incorporations.

When potash additions were made by means of red clover hay along with $Ca(OH)_2$, limestone and dolomite, there was produced a decided decrease in the outgo of the soluble potash.

As a whole, the results established the fact that the liming of rock-derived soils under humid conditions will depress the hydrolytic disintegration of both the original potassium complex and that formed by the fixation of soluble added potassium salts, and further that the protective, or buffering, effect becomes more pronounced with increase in concentration of the bicarbonates of calcium and magnesium.

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(*Received April 26th*, 1930.)

A NEW VOLUMETRIC METHOD FOR THE ESTIMATION OF SODIUM.

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The development during the last few years of reliable and accurate methods for the determination of the other soil bases has caused some dissatisfaction amongst soil chemists with the existing one for sodium. The complete separation of iron and the alkaline earths from the alkalies is performed only with great difficulty and generally involves repeated precipitations.

The modifications suggested by Husband and Godden(1) for the estimation of the alkalies in feeding stuffs ensure the more or less complete removal of calcium and magnesium, but too infrequently the residue is stained with iron. The doubt as to the absolute efficiency of the procedure is always present, and the gravimetric data finally obtained, especially when these are of the order of 10 mg. or less, are none too convincing.

The specific reagent used by Kolthoff(2) gives a precipitate with sodium, and a gravimetric method for the estimation of this alkali has been worked out by Barber and Kolthoff(3). Ammonium salts and small amounts of barium, calcium, or magnesium do not interfere and a concentration of potash equivalent to about six times that of sodium is without effect. Phosphates and organic acids interfere with the reaction.

Whilst the degree of accuracy attained by these workers leaves nothing to be desired, the manipulative details are somewhat troublesome. Some ten to fifteen washings are necessary in order to transfer the precipitate to the filter and to remove the last traces of the reagent. This procedure involves the risk of errors due to leaching even when the wash medium is saturated with the triple salt, to the introduction of extraneous matter, and to possible variation in the degree of hydration of the precipitate.

These drawbacks are obviated by the use of the proposed volumetric method, the principle of which is briefly as follows. The sodium is precipitated as uranyl zinc sodium acetate as in the gravimetric method just quoted. This is dissolved in dilute hydrochloric acid, reduced with titanous chloride solution to the uranous state, and stabilised or even

precipitated as uranous fluoride by the addition of hydrofluoric acid. The excess of titanous chloride is estimated by back titration with iron-alum solution using potassium thiocyanate as indicator. The amount of titanous chloride used can be expressed in terms of iron and uranium, and finally of sodium from a consideration of the formula of the precipitate given by Kolthoff ($(UO_2)_3ZnNa(CH_3COO)_9.6H_2O$).

1 Fe = 2·133 U = 0·06865 Na.
1 cc. $N/50$ $TiCl_3$ = 0·0000767 Na.

Preparation of the reagent.

Solutions required:

A.	Uranyl acetate ($2H_2O$)	10 gm.
	30 per cent. acetic acid	6 c.c.
	Water	60 c.c.
B.	Zinc acetate	30 gm.
	30 per cent. acetic acid	3 c.c.
	Water	32 c.c.

The salts in both *A* and *B* are dissolved by heating, the solutions mixed and allowed to stand for 24 hours, after which time any precipitate is filtered off. The reagent thus prepared is a practically saturated solution.

THE METHOD.

The solution containing the sodium preferably as the chloride, to an amount not exceeding 12 mg., is evaporated to dryness in a glass basin of about 20 c.c. capacity. 15 c.c. of the reagent are added and the whole well stirred and allowed to stand for 1 hour. It is then filtered through a 15 mm. diameter Jena glass filtering funnel (porosity G4) and as much as possible of the reagent removed by suction. The precipitate is then washed two or three times with about 2 c.c. each time of a saturated solution of the precipitate in 95 per cent. alcohol.

The uranyl zinc sodium acetate is dissolved in dilute HCl and transferred to a 500 c.c. conical flask. About 10 c.c. of strong HCl are added and the air in the flask displaced by passing a current of CO_2 gas. Excess of titanous chloride solution is then run in from a burette. Whilst still maintaining a current of the gas through the flask, the latter is shaken and allowed to stand for 2 or 3 minutes, or heated to 40–50° C. to ensure the complete reduction of the uranium. 20–25 c.c. of 2 per cent. HF are then added and about 10 c.c. of 10 per cent. potassium thiocyanate solution.

The excess of titanous chloride is estimated by back titration with iron-alum solution to the production of a permanent red coloration. It is generally preferable to add a slight excess of the iron alum and re-titrate with titanous chloride until the red colour disappears. The end point is sharp and accurate to two drops of the latter solution.

The hydrofluoric acid produces a bluish-green solution of uranous fluoride, which is stable and has no effect on the ferric salt. It is often found as an amorphous green precipitate at the end of the titration, especially if excess of HF has been used.

A convenient strength for the volumetric solutions is about $N/50$. The standardisation is most satisfactorily made with pure ferrous ammonium sulphate which has been carefully oxidised with potassium permanganate. (For full details as to the preparation and use of titanous chloride reference should be made to (4).)

$$1 \text{ c.c. } N/50 \ TiCl_3 = 0{\cdot}0000767 \text{ Na.}$$

EXPERIMENTAL.

Analysis of uranyl zinc sodium acetate.

A sample of the triple salt was carefully prepared from a solution of pure sodium chloride, the precipitate being finally washed with ether to remove the last traces of alcohol and water. The specimen showed no variation whatever in weight after being kept for 15 minutes on the balance. An accurately weighed amount was dissolved in water and made to 250 c.c. Aliquot portions were taken for ascertaining the amount of HF required to fix the uranous salt.

Table I.

A. Wt. of uranyl zinc sodium acetate = 2·2469 gm.
Made to 250 c.c. 20 c.c. taken.

	Sodium found	Sodium calculated
15 c.c. 1 % HF	0·00251	0·00269
20 c.c. 1 % HF	0·00256	—
25 c.c. 1 % HF	0·00267	—
40 c.c. 1 % HF	0·00270	—

B. Wt. of uranyl zinc sodium acetate = 2·0354 gm.
Made to 250 c.c., 20 c.c. taken. 20 c.c. 2 % HF.

Sodium found	Sodium calculated
0·00242	0·00243
0·00243	—
0·00242	—

In the above and subsequent calculations the weight of sodium is given by the following:

Wt. of sodium = wt. of uranyl salt/0·01495.

The results in Table I, *A*, show the necessity for the addition of sufficient HF to fix the whole of the uranous salt and those in Table I, *B*, confirm the accuracy of the above factor and the Kolthoff formula.

Application to mixed solutions.

For this purpose the following solutions were prepared from A.R. materials in order to simulate somewhat those likely to be expected in soil and plant ash extracts.

No. 1.	No. 2.
0·4321 gm. Na_2CO_3	0·4473 gm. Na_2CO_3
	0·4975 gm. KCl
No. 3.	**No. 4.**
0·4456 gm. Na_2CO_3	1·4706 gm. $CaCO_3$
0·4911 gm. KCl	0·2162 gm. MgO
0·0214 gm. $CaCO_3$	0·2038 gm. $FeCl_3$
0·0300 gm. MgO	0·1690 gm. $AlPO_4$

The compounds used were dissolved in dilute HCl and each solution made up to 1 litre, 10 c.c. being taken for the following determinations:

		Na_2CO_3	
Solution	Reagent c.c.	Found (gm.)	Calculated (gm.)
1	10	0·0041	0·0043
	15	0·0043	—
2	10	0·0044	0·0045
	15	0·0045	—
	20	0·0046	—
3	15	0·0045	0·0045
	20	0·0045	—

It is apparent from the above that the complete removal of the alkaline earths beyond, say, one precipitation with ammonium hydroxide and ammonium carbonate, is quite unnecessary in this method. Kolthoff and Barber found no interfering action with considerably larger proportions of the alkaline earths, and none with ammonium salts. Where the proportion of potash to sodium exceeds 5 to 1, appreciably greater quantities of the reagent should be used.

Separation of iron, aluminium, etc., by ignition.

10 c.c. each of solutions 3 and 4 were mixed, evaporated to dryness in a vitreosil dish and ignited for 30 minutes. The residue was extracted with water, filtered and well washed.

	Na_2CO_3	
Solution	Found (gm.)	Calculated (gm.)
4	0·00018	—
3 and 4 mixed	0·0032	0·0046

It would appear from this result that the loss of sodium by volatilisation with small residues is very appreciable, and that the time and nature of the ignition should be carefully controlled.

A gentle ignition for 10 minutes over a wire gauze was found sufficient to render the iron and phosphoric acid insoluble, and not to interfere with the content of sodium, *e.g.*

	Na_2CO_3	
Solution	Found (gm.)	Calculated (gm.)
10 c.c., No. 3	—	—
+10 c.c., No. 4	0·00464	0·00464

Separation of iron, aluminium, etc., by precipitation.

This was effected by a single treatment with ammonia and ammonium carbonate in aqueous solution, the filtrate being evaporated to dryness and the ammonia driven off by ignition. Allowing for the amounts present in the ammonia solutions and solution No. 4 the quantity of sodium found was in complete agreement with that calculated.

	Na_2CO_3	
Solution	Found (gm.)	Calculated (gm.)
10 c.c. ammonia + 10 c.c. ammonium carbonate	0·00011	—
10 c.c., No. 4	0·00018	—
15 c.c., No. 4 + 15 c.c., No. 3	0·00710	0·00706

SUMMARY.

1. A volumetric method for the determination of sodium which can be separated as a triple salt (uranium zinc sodium acetate) from mixed solutions is described. The reduction of the uranium with titanous chloride, upon which it depends is shown to be quantitative.

2. Calcium, magnesium, and relatively large proportions of potash do not interfere. The procedure for the removal of iron, aluminium and phosphates by gentle ignition is simple and obviates the risk of error by the introduction of sodium as an impurity in the chemical reagents necessary for precipitation methods.

3. 0·1 mg. of sodium can be determined accurately.

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(*Received April 26th*, 1930.)

NITRATE FLUCTUATIONS IN A SOUTH AUSTRALIAN SOIL.

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(With Five Text-figures.)

The fluctuation in the nitrate content of arable soils in southern Australia is of special significance in connection with the practice of bare fallowing as a preparation for wheat growing. In the wheat areas of Western Australia, South Australia, Victoria and the southern districts of New South Wales, fallowing is the recognised cultural basis for successful wheat production, and from a purely mechanical point of view the practice in the hands of the more skilful farmers has probably reached a degree of perfection which it would be difficult to surpass.

The beneficial effects of fallowing (1) have been attributed in general to the conservation of soil moisture from the fallow season to the succeeding wheat season, to the control of weeds and to the accumulation of nitrates. In addition, there is the important practical reason that it enables large areas of well prepared seed bed to be ready for the relatively restricted seeding season. The climatic conditions during a typical fallow in South Australia are of the Mediterranean type and may be summarised as a late summer period of intense dry weather from the stubble or grazing of the preceding season, a period of winter and spring rainfall during which the active cultivation of the fallows proceeds and during which the conditions for biological activity may be presumed to be satisfactory both with regard to soil moisture and temperature and a third hot dry period during which the surface layers of the fallow dry out, and all opportunities are taken to cultivate the soil after the occasional showers of rain. The desiccation of the soil mulch may possibly result in some simplification of the soil population akin to a partial sterilisation effect, but no evidence is available on this subject.

The moisture conservation aspect has probably received excessive prominence in the past and is under investigation at various centres in Australia at the present time. The purpose of the present investigation has been to determine the nature and importance of the fluctuations in the nitrate nitrogen content of the soil at the Waite Institute, Glen Osmond, South Australia, during the season under typical conditions of

cropping and fallowing. In addition a series of laboratory observations has been made on the nature of the nitrate fluctuations in this soil under different temperature and moisture conditions.

The soil of the Waite Institute consists of a very lightly leached soil probably of the brown earth type with well-marked horizons: the surface horizon consists of brown fine sandy loam, while the subsoil (B_1 horizon) is a heavy red clay. The reaction of the surface soil fluctuates about a mean value of *p*H 6·8.

Temperature and rainfall.

The soil temperature conditions at Glen Osmond are indicated in the following Table I, summarising average monthly temperatures for the five years 1925–29 during which the determinations recorded in this paper were made.

Table I.

Soil temperatures at a depth of 1 inch. Waite Institute, South Australia.
Mean monthly readings (° C.).

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1925	29·8	27·3	26·9	18·6	14·3	11·5	8·6	12·5	13·7	19·7	26·0	28·8
1926	30·3	29·3	27·7	20·9	12·3	10·7	10·0	10·4	14·8	17·8	24·2	26·3
1927	30·4	26·9	25·6	20·6	13·4	9·7	9·6	10·5	15·2	20·5	25·8	28·8
1928	27·8	24·9	23·7	20·4	12·7	10·2	9·4	12·6	14·6	15·8	23·1	27·6
1929	26·1	31·0	25·5	19·1	13·3	14·1	13·6	14·5	18·6	22·6	26·7	28·5
Mean	28·9	27·9	25·8	19·9	13·2	11·2	10·2	12·1	15·3	19·3	25·1	28·0

Soil temperatures at a depth of 6 inches. Waite Institute, South Australia.
Mean monthly readings (° C.).

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1925	26·0	23·4	22·8	16·4	12·5	10·4	7·5	10·4	11·6	15·9	21·9	24·8
1926	26·1	25·8	24·7	18·9	11·9	10·4	9·6	9·8	13·4	15·8	20·7	22·1
1927	25·6	22·7	21·3	17·6	12·7	9·2	9·2	9·9	13·4	17·3	21·5	23·8
1928	23·3	22·7	21·6	18·9	12·5	10·3	9·3	11·4	13·7	14·9	20·4	23·8
1929	23·3	26·3	22·1	17·3	12·7	13·4	12·8	13·3	16·6	20·2	22·8	23·6
Mean	24·8	24·2	22·5	17·8	12·5	10·7	9·7	11·0	13·7	16·8	21·5	23·6

The moisture content of the soil fluctuates with the season and with the cropping but there is evidently a considerable loss of water from the soil during the dry summer season in spite of the soil mulch on the surface of the fallows. The rainfall during the five years under observation is given in Table II. It may be noted that the three seasons, 1927–9, constitute what is probably a very unusual sequence of dry seasons for this locality. On the other hand, the rainfall is materially higher than is usually associated with the wheat belt of South Australia, and crop conditions have been relatively favourable. In 1929 a cool spring was a factor of considerable importance in the production of high yields.

Table II.

Rainfall at Waite Institute, South Australia. Monthly total rainfall (inches).

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Date of break of weather
1925	0·57	3·16	0·37	1·16	5·11	2·20	3·14	1·84	3·76	1·19	0·47	0·14	23·11	May 18
1926	Nil	1·08	0·01	1·83	6·27	2·14	2·29	4·71	2·76	1·91	0·79	1·97	25·76	May 20
1927	0·24	1·09	1·15	0·42	3·31	2·53	3·51	4·12	1·17	0·41	1·42	1·62	20·99	May 17
1928	1·09	2·63	1·36	1·36	2·65	4·58	3·33	0·84	2·04	2·97	0·53	0·14	23·52	May 25
1929	0·67	0·03	0·25	0·65	1·73	4·26	2·65	2·37	2·72	1·17	1·26	3·68	21·44	June 3

FIELD OBSERVATIONS.

Observations on the nitrate nitrogen content of the soil were made at regular intervals during the seasons 1925 and 1929, during the latter in considerable detail, and on occasion during the intermediate seasons. During the earlier observations samples were taken to a depth of 9 inches, but it became evident that fluctuations in the nitrate content with depth were of importance, and subsequent surface samples were taken to the depth of the soil mulch, normally to 3 or 4 inches. The soil samples were taken as speedily as possible to the laboratory and dried at 55° C. Duplicate 250 gm. lots were extracted to 500 c.c. on Buchner funnels and the extracts boiled for 2 hours with the addition of alkali. Magnesia was used for the uncoloured extracts of the deeper samples and 10 c.c. of 4 per cent. $KMnO_4$ and 5 c.c. of 30 per cent. NaOH for the coloured extracts of all the surface samples, and for those of the 4–9 in. samples which were collected during the wet season. A mixture of iron and zinc powders was used with soda to reduce the nitrates.

Condition of fallows at the break of the season.

A characteristic feature of the South Australian climate from an agricultural point of view is a definite break of the season, when the succession of rainy days and the amount of rainfall become satisfactory

Table III.

Condition of fallows prepared for wheat at the break of the season. Nitrate nitrogen (parts per million of dry soil).

Year	Remarks	Depth of soil in inches	Nitrate nitrogen
1925	Fallowed from virgin pasture, 1924	9	34
1926	Virgin pasture, 1924. Fallow, 1925	9	36
1926	Virgin pasture, 1924. Fallow, 1925	9	36
1928	Wheat, 1926. Fallow, 1927	9 (18)	33 (19)
1929	Wheat, 1927. Fallow, 1928	9 (18)	33 (19)
1929	Sown pasture, 1927. Fallow, 1928	9 (18)	37 (20)
1929	Peas, 1927. Fallow, 1928	9 (18)	35 (20)
1929	Virgin pasture, 1927. Fallow, 1928	9 (18)	28 (17)
1930	Wheat, 1928. Fallow, 1929	9 (18)	30 (19)
1930	Peas, 1928. Fallow, 1929	9 (18)	47 (30)

for the safe seeding of the winter cereals. Normally this takes place towards the end of May. At this time the fallows contain an amount of nitrate more than adequate for the subsequent needs of average wheat crops, but, as will be seen later, hardly adequate for the extraordinarily high yields obtained during the season of 1929. It is of interest at this stage to summarise the data obtained as a result of observations made at the end of the fallow period.

The data in general are averaged from a series of determinations made during the dormant season immediately prior to the late autumn rains, and indicate that the amount of nitrate accumulated during fallowing at the Waite Institute tends in general to a remarkable degree of constancy in the different seasons. Of particular interest is the result obtained after two years' fallowing during 1924 and 1925; in the first season thirty-four parts of nitrate nitrogen had accumulated in the surface 9 inches, while as a result of an additional season's fallowing the value is found to be the same. During 1929 the values in the fallow succeeding peas reached an unusually high value which will, in all probability, be reflected in the 1930 crop. The distribution of the nitrate at this period in the various soil levels is of particular interest; the bulk appears to be located in the surface mulch, the nitrate content of which can on occasion attain a remarkably high level. A few examples are given below.

Table IV.

Distribution with depth of nitrate in fallows.
Nitrate nitrogen (parts per million of dry soil).

Depth in inches	Autumn 1926		Autumn 1929		Autumn 1929	
	Nitrate	Moisture %	Nitrate	Moisture %	Nitrate	Moisture %
0–3½ (mulch)	72	2·3	73	4·5	52	1·5
3½–9	7	7·2	10	6·5	9	6·4
9–18	6	10·6	4	8·7	4	13·7
18–27	—	—	—	—	3	20·4
27–36	—	—	—	—	2	18·2

February 1930 (parallel samplings).

	Depth in inches	Nitrate	Moisture %	Nitrate	Moisture %
Mulch	0–1	65	1·3	58	1·1
	1–2	42	4·9	66	2·4
	2–3	34	7·9	20	6·7
Surface soil fine sandy loam	3–4	12	10·3	11	7·2
	4–5	9	10·0	8	7·9
	5–6	8	9·6	8	8·5
	6–7	9	9·9	7	8·5
	7–8	8	10·5	6	8·6
	8–10	7	10·7	5	9·2
Subsoil clay loam	10–12	6	11·0	4	11·6
	12–15	4	12·4	5	17·9

Similar data were obtained for the Veitch Experimental Farm in May 1929. The soil is much coarser in texture than at Glen Osmond and the rainfall very much lower, averaging 12 inches, but having had only 5·9 and 8·9 inches respectively in the two preceding seasons.

The data for Veitch given below show a much lower nitrogen level than in the region of higher rainfall, and owing to the scanty crop of the preceding season a relative high amount of nitrate in the stubble land.

Table V.

Nitrates in soils at Veitch, South Australia (parts per million of dry soil, May 1929).

Treatment	Depth 0–3½ in.	3½–9 in.	9–18 in.
Cropped to wheat 1928	14	13	13
Fallowed in 1928	24	12	—
Virgin mallee	10	17	6
Cleared 1928	14	8	7
Cleared for some years, fallowed 1928	37	12	9

In addition to this vertical distribution the relatively high value reached in the surface mulch suggested a possibly high degree of variability, and this was confirmed by a number of separate observations, examples of which are given below; the values refer to the nitrate nitrogen content, in parts per million of dry soil, found in single cores.

Plot 1. (Wheat, 1927. Fallow, 1928)	47	55	73	45	66	37
Surface mulch sampled, 8. iv. 29	14	23	25	56	69	68
Plot 7. (Sown pasture, 1927. Fallow, 1928)	30	58	84	36	74	62
Surface mulch sampled, 25. ii. 29	58	83	137	40	81	83
Plot 7. Mulch to 9 in., 25. ii. 29	12	8	20	11	14	16
	24	15	25	17	8	18

In most of the plot sampling during the observational work six holes were taken and united. The above observations correspond to standard errors of 16, 17 and 13 per cent. respectively, and it is evident that only major fluctuations in the nitrate content of the soil of any given plot can be taken to have particular significance. The plots had received uniformly careful treatment, and there is no reason to suppose that this high degree of variability is not a normal feature of nitrate distribution in fallow soils. In spite of this high variability a significant degree of correlation is frequently observable between the nitrate contents of parallel samplings of different fallow plots.

Nitrate content of rotation plots during the dormant season.

A series of permanent rotation plots at the Waite Institute afforded an opportunity for detailed study through the season. The data obtained over a series of observations made during the dormant season can be

averaged to give a more reliable estimate of the differences due to the different systems of cropping in the season of 1928. It will be observed that cropping has reduced the nitrate content to a low level. A comparison with data obtained after the 1925 crops shows that pasture is more exacting on the soil than wheat, and this again than peas.

Table VI.

Effect of cropping on the nitrate content of the soil during subsequent dormant period. Nitrate nitrogen (parts per million of dry soil).

Plot no.	Crop	Nitrate nitrogen: Mulch (0–3½ in.)	Nitrate nitrogen: 3½–9 in.	Nitrate nitrogen: 0–9 in.	Nitrate nitrogen: 9–18 in.	Moisture: 0–9 in. %	Moisture: 9–18 in. %
			Season 1925.				
1, 3, 5	Wheat	—	—	5·9	2·7	2·7	5·3
6	Sown pasture	—	—	2·8	1·5	5·3	5·6
4	Peas	—	—	10·6	5·8	3·6	6·2
			Season 1928.				
1	Bare fallow	57·0±3·0	17·2±1·6	33·0	5·6	2·2	8·6
2	Wheat	11·5±0·6	9·2±0·5	10·0	6·3	3·8	4·9
7	Bare fallow	77·0±5·0	11·5±0·7	37·0	4·5	4·5	8·7
12	Peas	18·3±2·0	12·0±1·5	14·5	7·0	3·5	6·5
13	Bare fallow	73·0±6·0	11·5±1·0	35·0	6·3	5·3	9·2
17	Wheat (continuously)	8·3	8·2	8·0	2·3	8·0	6·2

Seasonal fluctuations.

The fluctuations in the nitrate and moisture content of a number of plots were followed in some detail throughout the season and the data relating to nitrate is summarised graphically in Figs. 1 and 2. In Fig. 1 the high nitrate content of the surface mulch of fallowed land in the late dormant period March-April is well shown, the fluctuations observed at this period being probably due to variability in the field distribution.

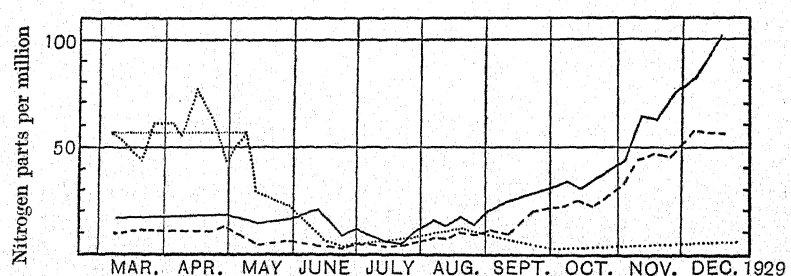


Fig. 1. Nitrate nitrogen content in surface 4 inches of field plots at Glen Osmond, South Australia, during the season.

Continuous line: Fallow after peas.
Broken line: Fallow after wheat.
Dotted line: Wheat after fallow.

The first effect of the rain is to wash down the nitrate from the surface soil and to redistribute this in the top 18 inches—later rains and the growing wheat crop prevent any further nitrate accumulation in the surface layer.

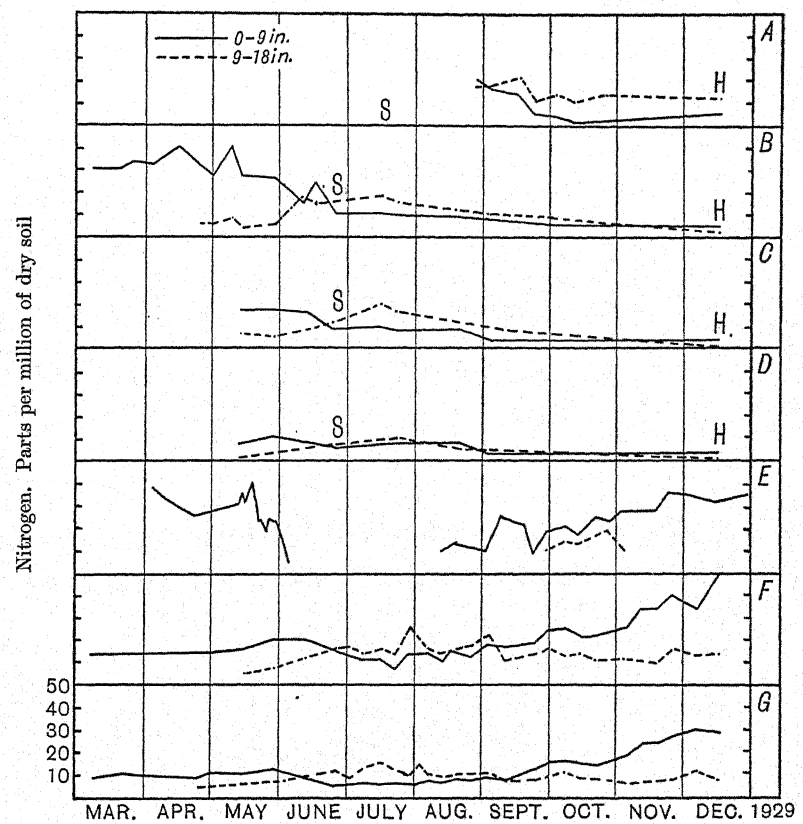


Fig. 2. Nitrate nitrogen content in surface 18 inches of various field plots at Glen Osmond, South Australia, throughout the season.

A. 1929. Border wheat plot sampled during actively growing period.
B. 1929. Wheat following bare fallow. Rotation plot 1.
C. 1929. Wheat following peas. Rotation plot 2.
D. 1929. Wheat continuously. Rotation plot 17.
E. 1925. Fallow following short fallow in 1924.
F. 1929. Bare fallow after peas. Rotation plot 12.
G. 1929. Bare fallow after wheat. Rotation plot 2.

S indicates seeding of wheat. H indicates harvest.

A similar redistribution is to be noted in the stubble plots which received their first cultivation on June 4, and henceforward became the fallows for the season. The first autumn rains appear to be much more

effective in leaching nitrate from the surface layers than do the winter and spring rains. In the latter cases the subsoil has presumably become sufficiently wet to hold up the additional soil moisture and to maintain the surface soil wet without permitting of the rapid percolation which is characteristic of the break of the season. From the end of July there is a steady accumulation of nitrate with minor fluctuations which are, however, parallel in the two separate plots and hence indicate something more than chance variation.

The rapid accumulation from mid-October on is more difficult to understand in view of the drying out of the soil. Two possibilities suggest themselves, either that nitrate accumulation proceeds fairly rapidly at low humidities in association with higher temperatures or that a certain amount of capillary redistribution has been effected. This latter is suggested by the fact that there is a slight fall in the nitrate content of the depth 9–18 inches in these later months. This steady accumulation remains still marked, however, if the results are averaged to a depth of 18 inches. The higher level maintained by the fallow prepared from pea stubble is also worthy of notice.

In the case of crops, the steady depletion in the nitrate is well marked. At no time, except possibly in early July, does nitrate accumulation proceed, and an examination of the yield data indicates that the actual rate of nitrate production under the crop was very much less than in adjacent fallows starting off under almost identical conditions. The following values, while only approximate, indicate the probable magnitude of these changes.

Table VII.

Effect of cropping on seasonal nitrate production
(parts per million of dry soil, 0–18 in.).

Plot	Crop	Rotation	Nitrate nitrogen in soil: At beginning of season	Nitrate nitrogen in soil: At end of season	Nitrogen in crop	Nitrate production during season
1	Wheat	Bare fallow, wheat	19	5	19	5
3	Wheat	Peas, wheat	12	4	15	7
17	Wheat	Wheat continuously	6	3	11	8
7	Wheat	Bare fallow, wheat, pasture	21	3	20	2
13	Wheat	Bare fallow, wheat, peas	21	4	21	4
E.B.	Wheat	Pasture, bare fallow, wheat	16	2	25	11
E.B.	Wheat	Pasture, wheat, wheat	5	2	13	10
2	Bare fallow after wheat		8	22	—	14
12	Bare fallow after peas		14	32	—	18

The reduced nitrification under a crop confirms the data that have been obtained elsewhere (Lyon, Bizzell and Wilson (2), Russell (3),

Prescott(4)) and it is unnecessary to discuss the matter further at this stage.

Soil conditions through the season.

In Table IV are given a few indications with regard to the moisture content of the soil during the dormant summer period. In general in summer, evaporation is so intense that even heavy rain does not keep the surface soil moistened for any considerable period, and light showers moisten the surface mulch for quite a restricted period of one or two days. The surface soil of the Waite Institute has a saturation value of about 24 per cent. calculated on the moist soil, and as will be seen later, would appear to have an optimum moisture content for nitrification of about 17 per cent. The factors likely to control nitrate accumulation are indicated in Fig. 3, where the observed soil moistures in fallows, and the temperature, evaporation and rainfall are given. The possibility of nitrate losses, presumably by micro-organic intake rather than by denitrification at certain periods when the moisture content exceeds the limiting value, is well shown. These losses take place apparently relatively rapidly with this soil type and probably acquire importance in the field at certain seasons of the year if the nitrate content of the soil is already high, although it is difficult to separate them from chance fluctuations.

Temperature conditions throughout are favourable to nitrification; the absence of a marked optimum as indicated in the laboratory experiments makes it difficult to select any period of the year when moisture and temperature conditions combine to give the most rapid rate of nitrate accumulation, but the field observations indicate the month of November with its falling moisture content and rising soil temperatures. Under a crop at this period the surface layers are very dry, and it is only in fallows that any considerable accumulation might be anticipated.

FACTORS INFLUENCING THE FLUCTUATIONS OF NITRATE IN THE SOIL.

The amount of nitrate in the soil at any moment is recognised to be the result of the balance between accumulation and depletion. Under fallow conditions the important nitrate depletion by growing plants is eliminated, but there is evidence that micro-organisms may play a part in this depletion under suitable conditions of moisture and temperature. The rate of nitrification in this particular soil might also be affected by the relative poverty in available phosphoric acid which is the most important

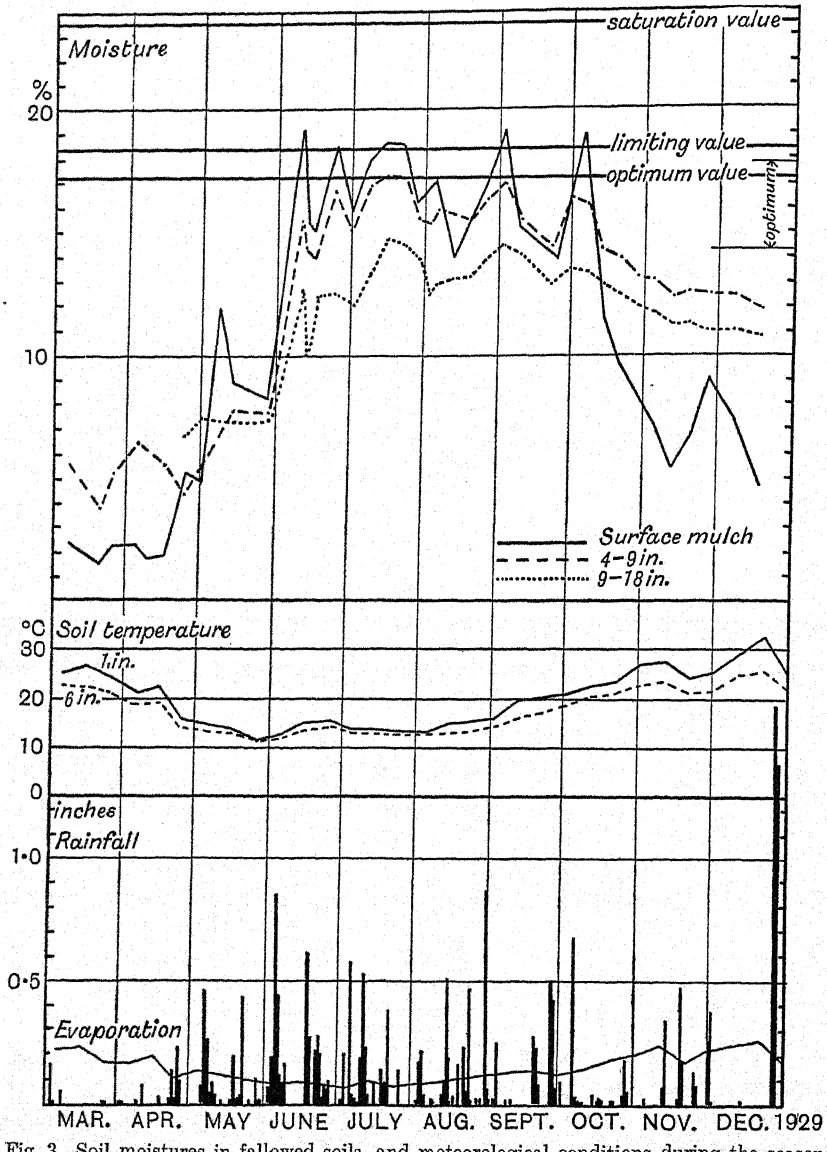


Fig. 3. Soil moistures in fallowed soils, and meteorological conditions during the season 1929 at Glen Osmond, South Australia. The optimum and limiting soil moisture values for active nitrate accumulation are indicated.

limiting factor in crop production. Lewcock(5) has already shown an increase in the activities of Azotobacter in South Australian soils by the enrichment of his cultures with phosphate solutions. The original nitrate content might also be presumed to have some effect on the final balance, and these points were investigated by a series of experiments in which the soil was moistened up to 14·7 per cent. water content and then stored for a period, the nitrate content being determined from time to time. In one series an amount of potassium phosphate equivalent to a dressing of 1 cwt. of 45 per cent. superphosphate per acre was added to the soil, while in another series a small amount of potassium nitrate was added. The results are tabulated below, it being assumed that the effect of the potassium would be of slight consequence on this soil.

Table VIII.

Amounts of nitrate nitrogen in stubble soil moistened up to 14·7 per cent. moisture content after addition of phosphate and nitrate (parts per million of dry soil).

Additions to soil	Nitrate present after					Nitrate produced in				
	0 days	30 days	62 days	97 days	183 days	0 days	30 days	62 days	97 days	183 days
Nil	3·0	13·6	33·7	40·0	60·0	—	10·6	30·7	37·0	57·0
Potassium phosphate	3·3	17·6	37·5	51·2	67·0	—	14·3	34·2	48·9	63·7
Potassium nitrate	42·3	45·9	70·2	88·4	105·0	—	3·6	27·9	46·1	62·7

The effect of phosphate in increasing the rate of nitrification is well marked, particularly in the early stages. The effect of nitrate as might be expected is more complex, and an initial slowing down of nitrification is followed by an increased rate after the first month.

Influence of moisture content.

In order to determine the effect of moisture content on the rate of nitrification, a series of this soil was moistened up to varying degrees of saturation and stored for a period of thirty-five days. In order to allow for the ready measurement of possible losses, a soil with an initially high nitrate content was selected, and to obtain a measure of the variability of the results five separate determinations were made on a soil stored for twenty-one days at a moisture content of 17 per cent.; in this case the individual values stood at 63·7, 68·2, 65·7, 66·5 and 69·3 parts per million of nitrate nitrogen, the original soil containing 56·7 parts. This range indicates that the minor fluctuations in the curves illustrated in Fig. 4 are of no particular significance. The optimum conditions for nitrification in this soil obviously occur at a moisture saturation value, as determined by the Keen-Raczkowski(6) method of 65 per

cent. or a moisture content of 16·7 per cent. calculated on the moist soil. Nitrification can evidently take place slowly at 20 per cent. saturation while there is evidently a sharp limit at 70 per cent. saturation beyond which conditions are favourable for the disappearance of nitrate.

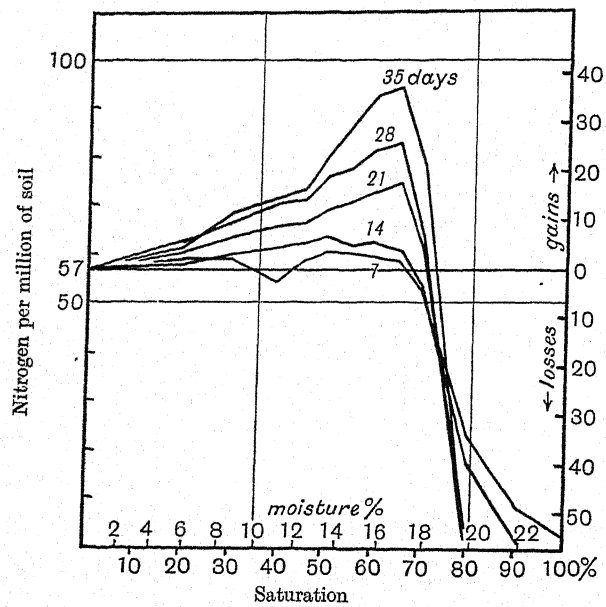


Fig. 4. Effect of soil moisture conditions on the rate of nitrate accumulation depletion.

The work of Subrahmanyan (7) with water-logged soils shows a limited loss of nitrate under these conditions, except in the presence of soluble organic matter, and the remarkably rapid disappearance of nitrate noted in our experiments would appear to demand further explanation. It may be noted that the general effects of the varying moisture content on the rate of nitrification are strikingly parallel to the data obtained by Greaves and Carter (8) with Utah soils.

Influence of temperature.

The effect of temperature was determined by moistening up a set of soils to a uniform moisture content of 14·7 per cent. and incubating in a multiple temperature thermostat allowing of fourteen different temperatures ranging from 4° C. to 40° C. The samples were distributed in fifty-six separate lots and were withdrawn for analysis at suitable intervals. The values obtained do not show a definite optimum tem-

perature except possibly after a relatively short period, but, over a period of forty-five days, incline to show a broad band of optimum conditions ranging from 11° C. to 35° C. which suggests either an exhaustion of available material or a complex process involving a series of separate reactions in which nitrate intake by organisms may be involved. In view of the fact that this particular soil sample accumulated up to fifty-seven parts per million of nitrate nitrogen when stored for 195 days, the second explanation would appear to be the more logical. The results of this experiment are summarised in Fig. 5. It is quite

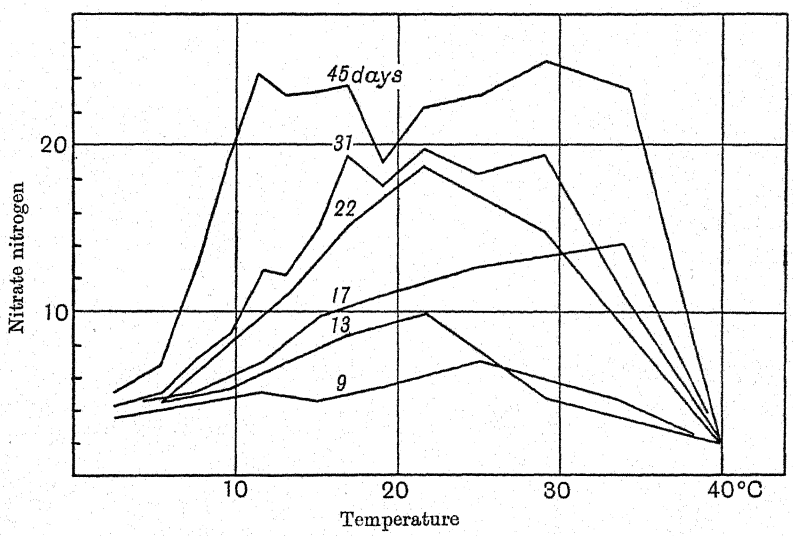


Fig. 5. Accumulation of nitrate in moist soil incubated at various temperatures. Nitrogen per million of dry soil. Moisture 14·5 per cent.

possible that a much more restricted temperature range for optimum nitrate accumulation might prevail under different moisture conditions, particularly with somewhat drier soils, but it is evident from the soil temperature conditions occurring naturally that at no period of the year is nitrification likely to be restricted by unfavourable temperature conditions.

GENERAL DISCUSSION AND SUMMARY.

The rate of nitrification in the soil under the conditions prevailing at the Waite Institute is governed primarily by the soil moisture conditions. The activity is greatest in the surface layers which are cultivated during the process of fallowing. Temperature conditions are generally favourable throughout the year, although there is evidence that the

high rate of nitrate accumulation in late spring, in spite of a rapidly falling moisture content, is associated with the higher temperatures prevailing at this period. The reaction to soil moisture conditions is much more sensitive—during the characteristic summer drought there is little evidence of change in the nitrate content of the soil, the quantities of nitrate accumulated during the preceding period of activity remaining unchanged. Moreover, laboratory experiments indicate that the possibility of any marked change occurring at this period will only exist in very exceptional seasons when the summer rainfall is considerably in excess of the average. The rate of nitrate production for the first few days under optimum conditions of temperature and moisture is relatively slow, and such conditions can only exist for a very small number of relatively brief periods during the summer drought period. The quantity of nitrate accumulated as observed over a succession of seasons would appear to approach a constant value of about twenty parts per million for the first 18 inches of soil. The greater portion of this nitrate remains in the first few inches of cultivated soil and is not washed down until the advent of the regular autumn rains, which appear to be more effective as leaching agents than any subsequent rains, probably owing to the initial dryness of the subsoil. Under the climatic conditions prevailing, there is no leaching down beyond the root zone of the cereal crops except possibly in seasons of exceptionally heavy rainfall. In one example, where the depth distribution to 36 inches was followed at intervals through a relatively dry season, there was little evidence of any material leaching of nitrate as far as the depth 27–36 inches. At certain periods of the winter the soil moisture content may be sufficiently high to result in the disappearance of nitrate, when present in quantity, from the surface soil, this depletion not being due to a leaching effect but possibly to an intake of nitrate by micro-organisms. Towards the end of the spring, with its period of rapid nitrate accumulation in the cultivated layer, two factors may be responsible, one a possible capillary redistribution due to rise from the lower layers, and a second due to particularly favourable moisture conditions immediately below the surface of the mulch or in the lower layers of the mulch itself—the effect of the mulch in delaying moisture losses may actually be more important in connection with this possible nitrate accumulation than in the conservation of moisture for the subsequent wheat crop.

During the biologically active period of the year the rate of nitrification under fallow conditions is appreciably more rapid than under a crop. In the particular season under intensive investigation, most wheat

crops grown on land previously fallowed barely found a sufficiency of nitrate for their needs. In one exceptional case, a 63-bushel crop, on fallowed land, recently broken in from pasture, an appreciable proportion of the nitrogen requirement of the crop was actually nitrified during the growing season. As has been observed almost universally elsewhere the rate of nitrate production in fallow was appreciably greater than in a cropped soil, after allowing for the nitrogen in the crop itself.

The field observations have been supplemented by a series of laboratory investigations with this particular soil stored under varying conditions of temperature and moisture content. Nitrate accumulation was favoured by a rather broad range of temperature conditions between 11° C. and 34° C. with no sharp optimum temperature, except possibly in the initial stages. There was in contrast a definite optimum moisture content (17 per cent.) for rapid nitrate accumulation with a rapid fall on the wetter side leading to nitrate depletion at moisture contents above 19 per cent. Nitrate accumulation occurred with relatively dry soil, although more slowly than under optimum conditions.

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(*Received May 23rd*, 1930.)

THE COLORIMETRIC DETERMINATION OF PHOSPHORIC ACID IN HYDROCHLORIC ACID AND CITRIC ACID EXTRACTS OF SOILS.

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Macromethods for the determination of acid soluble phosphoric acid in soils depending on its precipitation as ammonium phosphomolybdate suffer two disadvantages. In the first place the analytical operations—evaporation, ignition and quantitative filtration—are such that the time and lay-out involved are excessive. Secondly, the determinations sometimes show bad duplication. In some instances the precipitation is the source of error, while in others the ignition of the residue prior to the acid extraction of the phosphoric acid is the critical stage. Further, iron salts and the use of an ammonium molybdate solution, which has become insensitive through ageing, may prevent complete precipitation. For these reasons colorimetric methods should have the advantages not only of reducing the time of working, but of giving more accurate determinations for small amounts of phosphoric acid in acid extracts of soils.

I. Lonstein (1) applied the method of Denigès (2), which has been widely used for water extracts, to hydrochloric acid and citric acid extracts of a number of South African soils, and obtained good agreement with the gravimetric method. Briefly the treatment prior to developing the colour consisted of evaporating a small volume of the extract to dryness after the addition of calcium acetate solution. The residue was ignited to destroy the organic matter and to render the silica insoluble, and was then extracted with 10 per cent. sulphuric acid. After filtration excess acid was removed with ammonia and the colour developed with the ammonium molybdate and stannous chloride solutions.

Table I gives the results obtained by us for a number of English soils in a comparison of the gravimetric determinations as phosphomolybdic anhydride with colorimetric ones on extracts prepared by Lonstein's method, and using both the Denigès and Fiske-Subbarow reagents (3) to develop the colour.

These figures reveal the presence of some disturbing factor in the analysis of the heavier soils especially when the Denigès method of

colour production was used. The asterisk indicates that the blue colour developed slowly and had a green tint.

Table I.

		P_2O_5 per 100 gm. of soil					
		Hydrochloric acid extract			Citric acid extract		
Soil No.	Geological formation	Gravimetric	Denigès	Fiske-Subbarow	Gravimetric	Denigès	Fiske-Subbarow
A. 1262	Greensand	0·170	0·166	0·168	0·0501	0·048	0·049
		—	0·170	0·167	—	0·049	0·049
		—	0·165	0·168	—	—	—
A. 1263	Millstone Grit	0·076	0·069	0·073	0·0119	0·0117	0·0120
		—	0·071	0·074	—	0·0117	0·0119
		—	0·073	0·074	—	—	—
A. 1264	Red Clay loam over Lower Oolite	0·236	0·210*	0·221	0·0034	0·001*	0·0021*
		—	0·205*	0·216*	—	0·002*	0·0027*
		—	0·201*	0·209*	—	—	0·0015*
S. 761*b*	Clay loam with flints	0·132	0·117*	0·127	0·0124	0·008*	0·009*
		—	0·122	0·121*	—	—	0·008*
		—	0·120	0·124	—	0·005*	0·010*
S. 762	Clay loam with flints	0·195	0·175*	0·179*	0·0522	0·041*	0·045*
		—	0·170*	0·183	—	0·046*	0·047*
		—	0·183	0·182	—	0·040*	0·047*

In the analyses for which no results are reported in this table the green tint had become so pronounced that it was impossible to make comparisons.

Subsequently it was found that ferric salts added to a pure phosphate solution retarded and reduced the colour development, and gave the same green tint and fading that was observed during the analyses of the above soils. Ferrous salts had little effect. Further, the sulphuric acid extracts from the clay soils contained appreciable amounts of iron, while those from the lighter soils had only traces. This was regarded as additional evidence for the view that ferric iron was the interfering constituent. Attempts to reduce the amount of soluble iron by varying the time and temperature of ignition (using an electric muffle furnace) and the time of extraction with acid failed to give consistent results. While Matther(4) makes no mention of this difficulty when using the method of Fiske-Subbarow, Denigès(5) in a later paper overcame the interfering action of ferric salts by the use of metallic copper for reducing the iron and the ammonium phosphomolybdate. Belgrave(6) was unable to obtain satisfactory results by this procedure on Malayan soils, but obtained satisfactory results when zinc was used for the reduction of the iron and the colour developed in a solution of carefully controlled acidity.

Accurate colorimetric determination of phosphoric acid in soil extracts, therefore, demands not only the absence of large amounts of silica and organic matter and a controlled acidity, but also the absence of ferric iron. To satisfy these conditions a method was devised in which the organic matter, including citric acid, was oxidised by sodium permanganate in hydrochloric acid solution, the ferric iron precipitated by potassium ferrocyanide and the acidity adjusted by neutralisation with ammonia. As far as possible in this method lengthy operations such as quantitative filtration, dilution, evaporation and ignition were eliminated or reduced to a minimum. For this reason sodium permanganate with its higher solubility was used instead of the potassium salt for the wet oxidation process. The removal of any excess of oxidising substances, which was necessary for reasons discussed later, was found to be complete after a partial evaporation of the hydrochloric acid solution. In this way troublesome methods such as ignition or nitric acid treatment for the destruction of citric acid were avoided.

The procedure finally adopted for removing the iron was based on the following conclusions drawn from experiments on the behaviour of potassium ferrocyanide with iron, manganese, molybdenum, and aluminium salts when tested alone or in mixtures. Ferric ferrocyanide is very insoluble except in the presence of excess ferrocyanide. Manganese ferrocyanide is less insoluble than the ferric salt in acid solution but, at about the neutral point, it becomes so insoluble that the whole of the ferrocyanide can be removed in the presence of excess of manganese. Further, no manganese ferrocyanide is dissolved on subsequently acidifying the solution to about *p*H 3·0. Molybdenum gives a brown coloration and precipitate with ferrocyanide. Thus, by ensuring an excess of manganese and carrying out the precipitation of the iron with potassium ferrocyanide in acid solution and subsequently bringing the reaction to neutrality, it is possible to remove both iron and ferrocyanide completely and, in addition, to prevent any discoloration when the molybdate solution is subsequently added. This adjustment to approximate neutrality is readily made by utilising the fact that, on progressive additions of ammonia, the ferrocyanide precipitate changes colour from blue to bright purple at *p*H 6·8–6·9. It is, therefore, not necessary to add an indicator.

Since the phosphoric acid is also precipitated at this reaction, it becomes necessary to add sufficient acid to redissolve it. An upper limit to the amount permissible is set by the sensitivity of the colorimetric methods, especially Denigès, to acidity and the possibility of redissolving

the ferrocyanides. An acidity corresponding to *p*H slightly below 3 was found to be satisfactory, and can be reached most conveniently by adding a fixed amount of acid to the mixture so long as constant volumes of soil extracts and reagents are used. Spot tests with bromo-phenol blue can be used as a check or as an alternative.

With 1 per cent. citric acid solutions to which some iron and varying amounts of phosphoric acid (KH_2PO_4) had been added, the above treatment gave 99·3 per cent. recovery for 0·01–0·05 mgm. P_2O_5 by the Denigès method and 99·6 per cent. for 0·3–1·5 mgm. by the Fiske-Subbarow procedure.

The work of Parker and Fudge (7) on the effect of silica on the Denigès method and of Fiske and Subbarow on their own method suggested that interference from this source would not occur with citric acid extracts of soils. Confirmation of this was obtained from parallel tests with and without the removal of silica. In the case of hydrochloric acid extracts, however, the dissolved silica may amount to 0·2–0·9 per cent. and since in order to obtain a convenient depth of blue colour the volume of iron-free solution taken for colour development will vary with the concentration of phosphoric acid, it follows that interference by silica may occur with soils low in hydrochloric acid soluble phosphoric acid. Table II however shows that the effect is small except for soil S. 811*b*, where a value 20 per cent. higher is obtained when silica is not removed. Nevertheless the absolute error is small. It is therefore recommended that for soils with less than 0·02 per cent. P_2O_5 soluble in hydrochloric acid, complete evaporation to dryness and resolution with hydrochloric acid be substituted for the partial evaporation after the addition of sodium permanganate.

Table II. P_2O_5 *extracted by hydrochloric acid per* 100 *gm. of soil.*

	Denigès method		Fiske-Subbarow method	
Soil	SiO_2 removed	SiO_2 not removed	SiO_2 removed	SiO_2 not removed
S.	0·123	0·124	0·123	0·123
S. 805	0·191	0·193	0·190	0·191
S. 811*a*	0·030	0·030	0·030	0·030
S. 811*b*	0·011	0·013	—	—

The results of analyses in Table III of a variety of soils by the method finally adopted show very satisfactory agreement with the gravimetric method.

Table III.

Soil No.	Description	% P_2O_5 (HCl soluble) Method			% P_2O_5 (citric soluble) Method		
		Gravimetric	Denigès	Fiske-Subbarow	Gravimetric	Denigès	Fiske-Subbarow
A. 1262	Greensand	0·170	0·168	0·169	0·050	0·049	0·050
A. 1188	Moorland	0·049	0·048	0·050	0·0044	0·0042	0·0044
A. 1263	Millstone Grit	0·076	0·077	0·076	0·0119	0·0118	0·0119
S. 524*a*	Sandy loam	0·131	0·133	0·130	0·0210	0·0205	0·0205
S. 524*b*	Sandy loam	0·253	0·249	0·250	0·081	0·080	0·080
—	Fen	0·177	0·174	0·176	0·041	0·039	0·042
A. 1264	Red Clay loam (over Lower Oolite)	0·236	0·239	0·234	0·0037	0·0035	0·0036
S. 503	(Red Clay loam (over Lower Oolite)	0·124	0·126	0·123	0·0088	0·0085	0·0089
S. 497	Red Clay loam (over Lower Oolite)	0·102	0·101	0·099	0·0041	0·0042	0·0040
A. 1265	Clay with flints	0·087	0·085	0·086	0·0043	0·0045	0·0043
S. 761*b*	Clay with flints	0·132	0·130	0·131	0·0124	0·0120	0·0121
S. 762	Clay with flints	0·195	0·191	0·192	0·052	0·051	0·051
S. 761*a*	Clay with flints	0·247	0·243	0·245	0·062	0·063	0·061
—	Gold Coast	0·0135	0·0139*	0·0136*	—	—	—
—	Borneo	0·0066	0·0069*	—	0·00024	0·00026	—

* SiO_2 removed.

These methods are being applied to the determination of total and citric acid soluble phosphoric acid in basic slags. Table IV illustrates their value for the rapid analysis of phosphatic manures.

Table IV. % P_2O_5 *total.*

Method	Mineral phosphate	Basic slag		Superphosphate
	No. p. 119	No. p. 120	No. p. 121	No. p. 122
Gravimetric (pyrophosphate)	25·92	14·92	15·10	16·06
Colorimetric (Fiske-Subbarow)	25·7	14·4	15·0	16·1

It is believed that the low result for No. p. 120 is due to the interfering action of vanadium which produces a yellow colour in the solution by reaction with the potassium ferrocyanide.

In this work a Klett-Kober colorimeter was used, and the comparison was considered valid only if the colour ratio did not exceed 1·3. With a little experience of the Denigès or Fiske-Subbarow method the appropriate standard can be made from an inspection of the test 2 to 3 minutes after the reducing solution has been added. The colours of test solutions prepared by the procedure given in this paper develop at the same rate as the standards, whereas for those obtained by the ordinary method the rates are variable and always slower, even where the amount of iron was insufficient to affect the result. In consequence the selection of the

correct standard can now be made quite easily and quickly, and a dozen tests compared in half an hour from the time the solutions are developed.

Where a colorimeter is not available, Nessler tubes, graduated and with stopcocks, may be used with somewhat reduced accuracy. When this method is adopted the test solutions must be adjusted to the weaker end of the colour range. A 25 c.c. Denigès standard and a 3–4 c.c. Fiske-Subbarow standard are the maximum depths of colour that it is possible to compare in this way.

The final methods adopted for the determination of phosphoric acid in hydrochloric acid and citric acid extracts of soils are given below.

HYDROCHLORIC ACID EXTRACTS. (Using the A.E.A. 1905 method.)

20 gm. of powdered soil (1 mm. sieve) are placed in a flask, covered with 70 c.c. of concentrated hydrochloric acid and boiled for a short time. The flask is loosely stoppered and the contents allowed to digest in the water-bath for 48 hours. The solution is then cooled, diluted and filtered. This filtrate is made up to 250 c.c.

Destruction of organic matter and removal of iron.

15 c.c. of the extract are pipetted into a 100 c.c. conical flask and 0·5 c.c. of 20 per cent. sodium permanganate (1) added. The flask is then placed on a hot sand bath in a fume cupboard for about 15 minutes. By this time the contents should be simmering and free from any brown manganese precipitate. The liquid is cooled and diluted to about 30 c.c., 6 c.c. of 10 per cent. potassium ferrocyanide (2) are added, followed by 5 c.c. of 10 per cent. manganese sulphate solution (3) with frequent shaking of the contents. After standing several minutes the mixture is titrated with ammonia (4) until the blue colour just turns purple. 3·5 c.c. of 2*N* sulphuric acid (5) are added and the whole transferred to a 100 c.c. graduated flask, diluted to the mark and filtered. The first few c.c. are discarded. Where many analyses are to be made this transference may be avoided by using a 100 c.c. graduated flask throughout. Of the filtrate aliquots are taken for colour development by the Fiske-Subbarow or Denigès method given below.

Fiske-Subbarow. 10 to 50 c.c. are pipetted into a 100 c.c. graduated flask, diluted to 75 c.c. approximately, 10 c.c. of ammonium molybdate (6) added, then 4 c.c. aminonaphthol sulphonic acid solution (7) and the liquid made to the mark. The flask should be shaken during each

addition. The contents are finally poured into a 100 c.c. conical flask. 15 minutes later the test is compared with a standard made similarly from the standard phosphate solution (8).

Denigès. 1 to 25 c.c. are pipetted into a 100 c.c. graduated flask diluted to 90 c.c. and 1 c.c. ammonium molybdate (9) and three drops of stannous chloride solution (10) added, the flask being shaken between each addition. After diluting to the mark the contents are poured into a 100 c.c. conical flask, and compared after 5 minutes with a standard made similarly from the standard phosphate solution (11).

Citric acid extracts.

25 gm. of soil (through 2 mm. sieve) are placed in a half-litre bottle, and 250 c.c. of 1 per cent. citric solution added with extra citric acid equivalent to the calcium carbonate present and shaken in a mechanical shaker for 24 hours. The solution is then filtered. If the first portion of the filtrate is not clear it is returned to the filter.

Determination of organic matter and removal of iron.

75 c.c. are pipetted into a 300 c.c. Kjeldahl flask, 10 c.c. of concentrated hydrochloric acid added and followed by 12 c.c. 20 per cent. sodium permanganate (1). The sides of the flask are washed down with a little water. After standing half an hour the contents are vigorously digested till no manganese precipitate remains (about $\frac{1}{2}$ hr. more). The contents are transferred with a minimum of water to a 100 c.c. graduated flask. 4 c.c. 10 per cent. potassium ferrocyanide (2) are added slowly, drop by drop, with frequent shaking. Several minutes later the mixture is titrated with ammonia (4) until the blue colour just turns purple. 1·5 c.c. 2*N* sulphuric acid (5) are then added and made to the mark with water. After the solution has been filtered and the first few c.c. discarded, the colour is developed in an aliquot by one of the methods given under the analysis of hydrochloric acid extracts.

Where additional citric acid has been added for soils containing calcium carbonate, the amount of sodium permanganate should be increased proportionally.

Reagents.

(1) 20 *per cent. sodium permanganate solution.* 200 gm. of pure sodium permanganate are dissolved to 1 litre.

(2) 10 *per cent. potassium ferrocyanide solution.*

(3) 10 *per cent. manganese sulphate solution.* 50 gm. of $MnSO_4$, $4H_2O$ (A.R.) are dissolved to 500 c.c.

(4) *Ammonia* 1·1. 1 vol. of 0·880 ammonia (A.R.) is diluted with an equal volume of water.

(5) *2N sulphuric acid.*

(6) *Ammonium molybdate reagent.* (Dissolve 25 gm. of ammonium molybdate (A.R.) in 200 c.c. of water.) Rinse into a 1 litre graduated flask containing 500 c.c. of 1*N* sulphuric acid. Dilute to the mark.

(7) *Aminonaphthol sulphonic acid reagent.* Dissolve 0·5 gm. of aminonaphthol sulphonic acid 1:2:4 in 195 c.c. of 15 per cent. sodium bisulphite. Add 5 c.c. of 20 per cent. sodium sulphite and then further lots of 1 c.c. with shaking until solution is complete. This reagent will keep for a week in a stoppered bottle.

(8) *Standard phosphate solution.* Dissolve 0·1917 gm. KH_2PO_4 (A.R.) in 1 litre.

1 c.c. = 0·0001 gm. P_2O_5.

Addition of toluene to the solution is recommended.

(9) *Ammonium molybdate reagent.* Add 100 c.c. of 10 per cent. ammonium molybdate solution to a mixture of 150 c.c. water and 150 c.c. concentrated sulphuric acid (A.R. and arsenic free). The reagent should be kept in the dark.

(10) *Stannous chloride solution.* To 0·1 gm. of tin foil add one drop of a 4 per cent. copper sulphate solution and 2 c.c. of concentrated HCl. When reaction is complete dilute to 10 c.c. and filter. The reagent must be prepared freshly each day.

(11) *Standard phosphate solution.* Dissolve 0·1917 gm. KH_2PO_4 (A.R.) in water and dilute to 1 litre. Take 10 c.c. and again dilute to 1 litre.

1 c.c. = 0·000001 gm. P_2O_5.

Addition of toluene to the solution is recommended.

NOTES.

1. For peaty soils 2 c.c. of 20 per cent. sodium permanganate should be used for the oxidation of the hydrochloric extract.

2. For soils with less than 0·02 per cent. P_2O_5 soluble in hydrochloric acid the solution after oxidation should be evaporated to dryness, taken up with 2 c.c. concentrated hydrochloric acid and water and the iron then precipitated without filtering off the silica.

3. Ferricyanides must not be present, as the solution after the precipitation of the iron will be coloured. Oxidising substances, therefore, must also be absent.

4. The addition of the stated amounts of 10 per cent. sulphuric acid to the mixture of ferrocyanides produces an acidity neither excessive, after taking aliquots and diluting, for colour development nor too low for solution of the phosphate. If necessary one drop may be withdrawn, and a spot test on a filter paper with bromo-phenol blue should give a full yellow colour.

The same drop will also serve for a ferric iron test.

5. The range of standards for Fiske-Subbarow method is 2 to 15 cm. of the standard phosphate solution and that for Denigès is 5 to 50 c.c. For colorimeter work the deeper colours are preferable.

6. The factor 0·995 was used in the results given to correct for the volume of the ferrocyanide precipitate.

SUMMARY.

1. The colorimetric determination of phosphoric acid in hydrochloric and citric acid extracts of soils by a method involving the evaporation of the extract, ignition and acid extraction of the residue with either the Denigès and Fiske-Subbarow methods of colour development was satisfactory only with light soils.

Clay soils gave low results owing to the presence of larger amounts of iron.

2. A method is given in which the organic matter and iron are removed by treatment with sodium permanganate and potassium ferrocyanide. The results are in good agreement with the gravimetric method.

ACKNOWLEDGMENT.

One of us (A. J. P.) desires to thank the Ministry of Agriculture for a Scholarship, which enabled him to co-operate in this work.

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(*Received May 13th*, 1930.)

"SINGLE VALUE" SOIL PROPERTIES: A STUDY OF THE SIGNIFICANCE OF CERTAIN SOIL CONSTANTS.

V. ON THE CHANGES PRODUCED IN A SOIL BY OVEN DRYING.

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(With One Text-figure.)

One of the commonest physical measurements made on a soil is the determination of its air-dry moisture: the soil, after being allowed to attain equilibrium with the laboratory atmosphere, is heated, either for 24 hours or overnight, in a water oven, and the loss in weight is taken as a measure of the water content of the air-dry soil. As pointed out in Paper I of this series (1), such a measurement is liable to various sources of error; the results will be affected, to greater or less extent, by the temperature and humidity of the atmosphere in the laboratory and in the interior of the oven. In the present work, attention is confined mainly to the effect of the temperature of the oven, and errors due to variations in the atmospheric humidity are eliminated by using *hemihygric*[1] soil.

When the air-dry moisture is determined by the method referred to above, the assumption is made that, at the temperature of the water oven, the water contained in the soil (other than constitutional water) is removed, while other soil constituents are unaffected. Since the greater part of the water in the air-dry soil is held in the soil capillaries (1) under reduced vapour pressure, it is improbable that all the soil water will be removed under the conditions prevailing in the water oven. An attempt is made in the following work to measure the effect produced by alterations in the temperature of the oven, and to determine what physical significance can be attached to the air-dry moisture. Measurements of this kind are mentioned in a paper by Puri, Crowther, and Keen (2), but in that paper attention is paid more particularly to the effects of changing humidity than to those of different temperatures.

[1] To avoid frequent repetition of the phrase "atmosphere at 50 per cent. relative humidity," this will be called a *hemihygric* atmosphere. A soil in equilibrium with this atmosphere is termed a *hemihygric* soil; and its moisture content, measured by drying over concentrated sulphuric acid (*R* in Papers I and II) is the *hemihygric coefficient*. I am indebted to Prof. A. Petrie (Professor of Classics in this College) for this term.

EXPERIMENTAL TECHNIQUE.

Fisher and Jones (3) have carried out an extensive series of measurements on the moisture content of wheat and of flour. Their results showed that, in replicate experiments, there were fairly large discrepancies. These discrepancies were most pronounced when the drying was carried out in a water oven of the usual type, and were considerably reduced when an electric oven was used. In the present work, a large electrically controlled Hearson oven, of internal dimensions 15 in. × 15 in. × 22 in. was used; the amplitude of the temperature fluctuation was about 1°.

In order to test the accuracy obtainable, twelve replicate measurements were carried out on the same soil, of which samples were heated for successive days at various temperatures, ranging from 100° to 250°. In this experiment, the samples were contained in small petri dishes, and on each day the dishes were placed in the same positions in the oven. Subsequent examination of the deviations of each sample from the mean showed that there was no correlation between the results and the positions of the dishes in the oven. But it was found that, in general, the results from a given sample at different temperatures showed a consistent tendency to be either higher or lower than the mean. It is probable, therefore, that the main source of the observed discrepancies lies in the original sampling. The results are summarised in Table I, which shows the percentage loss in weight, w, with its probable error[1] after heating to the specified temperature, T.

Table I.

T	w	P.E.
100	3·709	0·002
105	3·814	0·003
110	3·861	0·002
115	3·933	0·002
121	3·962	0·002
132	4·201	0·002
144	4·342	0·002
153	4·512	0·002
163	4·731	0·003
179	5·355	0·003
191	5·740	0·004
203	6·142	0·004
212	6·512	0·004
226	6·976	0·004
237	7·445	0·004
251	7·758	0·004

[1] $\frac{2}{3}\sqrt{\frac{\text{(Sum of (deviations)}^2\text{)}}{132}}$.

It should be mentioned that the small probable errors give an unduly favourable estimate of the accuracy of the results, since they do not make any allowance for temperature fluctuations (all the samples being affected in the same way by such fluctuations). In subsequent work, with smaller numbers of replicates, much greater probable errors were found, but these present results are quoted to show the degree of accuracy attainable with the technique if necessary. For the purposes under consideration, it is sufficient to obtain results reliable to within 1 per cent. of the true value, and all the data used in the construction of Fig. 1 below fulfil this condition. For example, the greatest error in the whole series, obtained with Soil no. 56 at 192°, gave $w = 12{\cdot}97 \pm 0{\cdot}12$.

Four soils, of widely different types (nos. 15, 41, 46 and 56, described in Paper II(4)), were examined in detail as regards their behaviour on heating in the oven. The technique adopted was as follows: Samples of about 5 gm. of the hemihygric soils were weighed out. In one set of experiments the samples were contained in small petri dishes; in a second, independent set, squat weighing bottles with ground glass lids were used. There were no appreciable differences between the two sets of results: Fig. 1 shows the results for the second series. The samples were heated for successive days to different temperatures in the oven. It was found that 22 hours was a sufficient period for the heating. The soils were placed in the hot oven at 11 a.m. on the first day, and transferred to desiccators at 9 a.m. on the second day. After cooling and weighing, they were replaced in the oven at 11 a.m., the temperature was raised by a suitable amount, and they were then left until 9 a.m. on the third day—and so on, until the final temperature was about 250°.

Results.

The results are presented in Fig. 1, which shows the percentage loss in weight for each of the soils over a temperature range from 50° to 250°. The points marked with black dots represent means of six replicates for Soil no. 15 and of five replicates for Soils nos. 41, 46 and 56.

An inspection of the figure shows at once that there is no discontinuity in the curves in the region of 100°, as would be the case if the air-dry moisture corresponded to the complete removal of free or interstitial water from the soil. The curves are smooth throughout the temperature range examined, but each shows two points of inflexion, corresponding to maximum and minimum values of dw/dT. The maxima occur in the neighbourhood of 200°, but cannot be judged with any great accuracy, as the curves are nearly rectilinear between about 180° and 220°. It is

noteworthy that the minima do not occur at 100°, but at temperatures which, for the four soils, vary from 100° to 120°. Moreover, these minima do not occur at the moisture content represented by the hemihygric coefficient, which has been shown in Papers I and II to be intimately associated with the interstitial water. The conclusion drawn from these

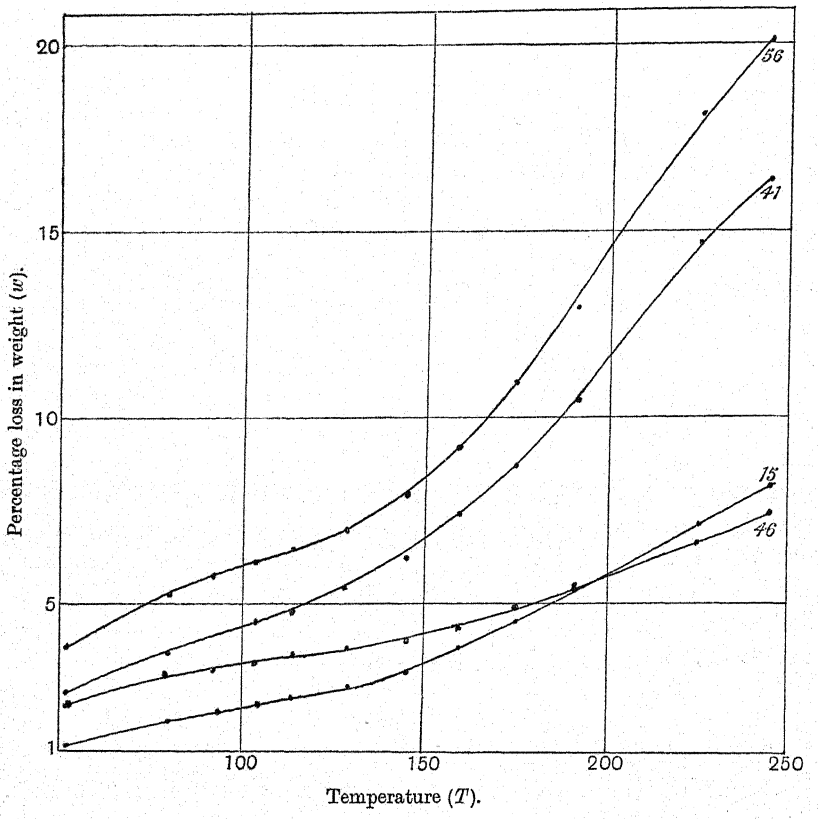


Fig. 1.

results is that no fundamental change in the structure or properties of the soil occurs abruptly when it is heated to 100°, and that the air-dry moisture can only be regarded as a convenient empirical number, not representing any special physical property of the soil.

A question of interest for routine practice may be discussed at this point. If there is no particular virtue in choosing 100° for the determination of the air-dry moisture, it is worth while considering to what extent the results are affected by the lower temperatures generally

obtained in a water oven. A numerical example will illustrate the point. At Pietermaritzburg (2200 ft. above sea-level) the boiling point of water is usually about 98°, and the temperature in a water oven is 95° or 96°, say 5° below the standard boiling point. Table II shows the (approximate) average values of dw/dT covering this range, and the value of w at 100° for the four soils mentioned above, obtained by interpolation on the curves.

Table II.

Soil	15	41	46	56
dw/dT	0·016	0·033	0·014	0·036
w at 100°	2·24	4·40	3·40	5·02

These figures show that an alteration of 5° in T leads to a change of about 4 per cent. in the value of w—a figure which is probably not greater than the difference between duplicate determinations in an ordinary water oven. It may be concluded, therefore, that for approximate measurements (which are the only ones for which the air-dry moisture has any value) the air-dry moisture can be determined by the method used in ordinary routine practice. It has been shown earlier (in Paper I) that, for cases where greater accuracy is required, the air-dry moisture is of less significance than the hemihygric coefficient.

Although the errors indicated by the results shown in Table II are unimportant so far as measurements of air-dry moisture are concerned, they may be of considerable importance in some other measurements depending on oven drying, *e.g.* in the determination of the clay fractions in mechanical analysis. The official method of mechanical analysis specifies drying of the samples at 105°, and the data given above suggest that considerable errors will be introduced if the oven is not maintained very near to this temperature.

Another point to which attention is drawn by these data is the specification of a "dry" soil; evidently, a soil "dried" in a water oven is a somewhat uncertainly defined quantity. For this reason, it is suggested that when any data are to be expressed as percentages of a soil, it will be most satisfactory, and free from uncertainty, if the quantity is referred to either the hemihygric soil, or to soil dried over concentrated sulphuric acid, as a basis. The data in Table I of this paper are given on the former basis.

SUPPLEMENTARY DATA.

Further insight into the changes taking place when a soil is heated can be obtained by considering the results of the following experiment:

Samples of about 5 gm. of the hemihygric soil were placed in the oven. After one day, one sample of each soil was withdrawn; its loss in weight was determined, and it was then transferred to a vacuum desiccator with a hemihygric atmosphere. The temperature of the oven was raised, and on the second day another set of samples was withdrawn and treated in the same way. On five successive days, samples were obtained in this manner that had been heated to 63°, 90·5°, 116·5°, 147·5° and 222°. When all the samples had attained equilibrium with the atmosphere in the desiccator, they were weighed again, and the regain in weight determined. It was expected that the process of rewetting would be slow, but after the soils had been in the desiccator for a week no significant change in weight occurred in a further 10 days. The results are shown in Table III, in which w gives the percentage loss in weight on heating, and w' the subsequent regain.

Table III.

Soil no.	 15			41		
T	w	w'	$w - w'$	w	w'	$w - w'$
63·0	1·66	0·98	0·68	2·92	1·93	0·99
90·5	2·02	1·15	0·87	3·89	2·41	1·48
116·5	2·55	1·36	1·19	4·88	2·79	2·09
147·5	3·06	1·11	1·95	5·80	2·37	3·43
222·0	7·03	1·29	5·74	13·40	3·01	10·39
Soil no.	 46			56		
T	w	w'	$w - w'$	w	w'	$w - w'$
63·0	2·22	1·47	0·75	4·09	2·51	1·58
90·5	2·73	1·88	0·85	5·28	3·03	2·25
116·5	3·56	2·45	1·11	6·40	3·48	2·92
147·5	3·75	2·11	1·64	—	—	—
222·0	6·10	2·21	3·89	16·30	3·47	12·83

From Table III it will be seen that there are very marked differences between w and w'; these differences appear to be much greater than the hysteresis effect discussed by Puri, Crowther, and Keen (2), obtained by successive drying and wetting of a soil under isothermal conditions. It should also be noticed that as the temperature is raised the recoverable loss, w', changes but slightly, while the total loss, w, increases rapidly, as already shown in Fig. 1.

These results can be satisfactorily explained by supposing that the recoverable loss is due to the removal of free and interstitial water, while the irrecoverable loss is accounted for partly by the removal of water bound adsorptively or chemically by the soil colloids, and partly by the destruction of organic colloids, the last factor becoming increasingly important as the temperature is raised higher. The partial decomposition

of the colloids, by the removal of their bound water even at moderate temperatures, gives an adequate explanation of the smaller amount of water that the soil can retain in the "regain" experiment above.

Puri, Crowther, and Keen (2) give results for the losses in weight of a heavy subsoil (soil E, Hoos 1.0, clay 24·6 per cent.) on heating to 200°. They found that the loss in weight on heating from 100° to 200° showed a regular linear increase with increasing temperature, from which they derive "strong circumstantial evidence that the main effect of heating (up to at least 200° in this instance) consists in the removal of strongly held water from hydrous substances and produces no great alteration in the fundamental structure of the soil." In the cases described in this paper, it appears probable that the structure of the soils is attacked at a considerably lower temperature. The destruction of the colloids in comparatively large amounts is probably represented by the rapid increase in dw/dT shown by the steep upward trend of the curves in Fig. 1 between about 130° and 170°. Over this region it is probable that the loss in weight is made up of water removed from the fine capillaries and also by the destruction of colloids. The decrease in dw/dT at higher temperatures indicates that practically all the interstitial water has been removed, and that further losses are due almost entirely to the destruction of colloids.

The theory advanced here explains at once why there is no temperature at which a sudden break occurs in the curves in Fig. 1. It explains also the well-known fact that an oven-dried soil shows a tendency to cake, and that it is difficult to secure dispersion of such a soil. The writer has suggested elsewhere (5) that there is a close connection between the inorganic soil colloids and the clay as measured in mechanical analysis; destruction of part of the inorganic colloids, by heating to moderate temperatures, will, on this view, lead to a reduction of the clay measured by the ordinary methods of dispersion[1].

These conclusions confirm the views to which we have been led by the work described in earlier papers of this series with reference to the classification of soil water. While it is convenient to regard the soil water as being made up of free, interstitial, and chemically and adsorptively bound water, these terms must only be used in a broad general way: there is no justification for supposing that there is any sharp line dividing one class of water from the others, or that in any process one of the classes of water will be removed, while the others are unaffected. In any actual

[1] The loss of bound water is probably one of the factors operative in the "poaching" of a soil when dried in hot sunny weather after rain.

process all the water will play a part although, of course, under suitable conditions, one class may play a far more important part than the others. For example, the hemihygric coefficient is controlled mainly by the interstitial water.

The arguments developed from the data in this paper strengthen the opinion which the writer has advanced elsewhere (5) that errors in the estimation of organic matter (*i.e.* organic colloids) from loss on ignition figures are accounted for by the fact that the loss on ignition includes a considerable proportion of bound water.

SUMMARY.

1. It is shown that results for the loss in weight of a soil on oven heating can be obtained to a very satisfactory degree of accuracy when a Hearson electrically controlled oven is used.

2. Results obtained by heating soils to temperatures ranging from 50° to 250° give smooth curves connecting loss in weight with rise in temperature; from which it is concluded that there is no sudden alteration in the structure of a soil when it is heated to 100°, and that the air-dry moisture of a soil, as determined with sufficient accuracy by the usual methods, is a convenient empirical factor, but not a representation of any fundamental soil property.

3. An examination is made of the factors contributing to the observed total loss in weight when the soil is heated, and an explanation offered of the contributions made by the different types of soil water and by the soil colloids. It is found that the conclusions drawn from this discussion confirm views developed earlier with regard to the behaviour of the water in the soil, and the absence of any sharp dividing line between the different classes into which the soil water is usually divided.

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(*Received May* 21*st*, 1930.)

THE DECOMPOSITION OF UREA IN SOILS.

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The decomposition of urea in soils is a microbiological process of some importance in agriculture. In most systems of animal husbandry combined nitrogen is largely returned and added to soil in urea. During the ammonification of calcium cyanamide urea is formed as an intermediate product, and it may also be produced during the decomposition of proteins by micro-organisms (11, 12, 13, 14). In recent years the industrial manufacture of urea from atmospheric nitrogen has increased its importance as a nitrogenous fertiliser.

It has been shown (1, 2, 17, 22, 26, 28) that in various solutions inoculated with soil urea is rapidly converted to ammonium carbonate. The decomposition in soil itself has also been studied quantitatively (4, 5, 6, 7, 15, 16, 18, 19, 23, 27) but only a small number of soils have been examined in this way. In most of the experiments the urea was decomposed very rapidly. Nitrification tests (15, 18, 25) and numerous manuring trails have also indicated that urea is rapidly decomposed in most agricultural soils. It has been stated (20), however, that urea-decomposing bacteria may be absent from various soils, and it appeared to be possible that the slow availability of calcium cyanamide in peaty soils is attributable to the inactivity of these organisms.

In this work the decomposition of urea has been investigated in a wide range of soil types. An attempt has been made to carry out a general survey rather than to demonstrate fine distinctions between particular types of soil.

Methods.

With a few exceptions which will be specified the following methods were used.

Soil samples. The samples were taken from the layer about 3 to 5 inches below the surface over an area of about 1 sq. foot. Aseptic precautions were found to be unnecessary. The soils were passed through a 3 mm. sieve, thoroughly mixed, and used for the biological tests in an undried condition as soon as possible after being brought to the laboratory.

Solution tests. The medium used was soil extract (1 kg. garden soil and 1 litre tap water autoclaved to $1\frac{1}{2}$ atmospheres, filtered and the filtrate made up to 1 litre) + 0·05 per cent. K_2HPO_4 + 5 per cent. urea. The soil extract was all made at the same time. After being thoroughly mixed it was distributed in flasks which, after sterilisation, were capped to prevent evaporation and were stored until required. Weighed amounts of unsterilised urea were placed in sterile 300 c.c. Erlenmeyer flasks, and just before inoculation 50 c.c. of nutrient solution were added to each flask by a sterile pipette. A 5 gm. quantity of fresh soil was used as inoculum, and the flasks were incubated for 48 hours at approximately 30° C. on a shelf of an incubator which maintained an even temperature. After incubation the ammonia in 1 c.c. quantities of the cultures was titrated against $N/14$ H_2SO_4, the contents of the flasks being first properly mixed. A correction for the titre of 1 c.c. of fresh medium has been applied to the figures presented. Uninoculated but incubated controls seldom showed an appreciable decomposition in the incubation period used.

Soil tests. It was found to be necessary to use a very short incubation period and to add a comparatively large quantity of urea to the soils in order to bring out differences in urea-decomposing activity. Amounts of soil corresponding to 25 gm. of oven-dry material were placed in Petri dishes and a quantity of urea solution containing 250 mg. of urea was then distributed over the soil from an accurate burette. The standard urea solutions were always freshly prepared from pure, dry urea. The moisture content of each soil was brought to 65 per cent. of its water-holding capacity, distilled water being added if necessary. The covered dishes were placed singly on an incubator shelf where the temperature was 22° to 23° C. After 24 hours' incubation the undecomposed urea was extracted from the soils and determined by a urease method which has already been described (9). Control experiments on the recovery of added urea have been carried out with a great variety of soils, the same quantities of soil and urea being used as in the decomposition tests. For all the soils examined the percentage of urea recovered never fell below 97 except in the case of peats, and only when the urea was extracted from peat by the method of leaching in a Buchner funnel. The results obtained in a number of tests with different samples of peat, where the peat was leached in a Buchner funnel, can be summarised as follows:

Amount of peat (dry matter) used (gm.)	25	12·5	6·25
Percentage of added urea (0·25 gm.) recovered	90–92	94–97	96–98

The low recovery from 25 gm. (equivalent to about 120 gm. of fresh)

peat is attributable to the sponge-like nature of the material which makes it impossible to replace the absorbed water during filtration. It was concluded that urea undergoes no absorption in any soil.

Soil reaction. The *p*H of most of the soils was determined by the quinhydrone electrode (3). The colorimetric drop ratio method (10) was employed for certain alkaline soils.

Loss on ignition. The values quoted are percentages of the oven-dry weights of the soils.

Experimental error. All the figures to be presented are averages of two determinations, which, in the majority of cases, gave closely agreeing results. The main source of error in the biological tests was probably, however, a variability of incubator temperatures, and this is not reflected in the degree of agreement between duplicate tests, as in most instances these were made simultaneously in the same incubator. A measure of the variability of results secured at different times was obtained by frequent tests with soil from one plot. The standard deviation (8) of the results of 76 solution tests was nearly 7 per cent. of their mean. The results of 56 soil tests with soil from the same plot showed a standard deviation of a little over 10 per cent. of their mean. In the comparisons of arable, pasture and forest soils from each location the tests were made concurrently so that the chief source of error was probably avoided in each comparison.

THE DECOMPOSITION OF UREA IN MOUNTAIN AND HEATH SOILS.

Large areas of Scotland are occupied by natural pastures which, for a variety of reasons, are relatively infertile. Examples of such poor pasture soils occur on the hill section of the College farm at Boghall. The decomposition of urea has been investigated in some of the more distinctive soil types from this source. The average results obtained with six different samples of each are given in Table I along with short notes on the soils. The chief characteristics of the herbage have been included to give an indication of the soil conditions.

The samples of waterlogged soils (Type A) were partially dried before tests were made, and for soil tests they were incubated at 65 per cent. of their water-holding capacity. The figures obtained indicate that the micro-organisms of this soil are capable of decomposing urea rapidly. Soil tests were also made under strictly anaerobic conditions in a McIntosh and Fildes jar. For most of the samples the amount of decomposition which occurred anaerobically represented over 70 per cent. of the aerobic decomposition.

Table I. *The decomposition of urea in mountain and heath soils.*

Soil and herbage types	*p*H	Loss on ignition	Urea decomposed % In solution	Urea decomposed % In soil
Type A				
Soil. Glacial drift, permanently waterlogged by seepage water; generally dark in colour. The low acidity of the soils is associated with the percolation of waters carrying bases from subsurface strata.	6·1	11·7	58	65
	5·3	18·3	44	60
	6·3	15·6	70	40
	6·0	10·6	58	46
	6·0	13·1	30	89
Herbage. Predominantly rushes and sedges; varying proportions of other plants.	6·2	14·0	58	60
Type B				
Soil. Brown, sandy loam, either glacial drift or hill-wash derived from basalts or basic andesites; freely drained; dry during most of the year.	4·7	16·2	32	40
	5·2	14·4	31	42
	4·8	16·8	51	97
	5·0	15·6	55	95
Herbage. Mainly *Festuca ovina*, to a smaller extent *Agrostis vulgaris*, forming a short dense turf with an abundance of fibrous roots. *Ulex* becomes dominant if not destroyed.	4·7	17·3	49	92
	4·9	14·9	27	91
Type C				
Soil. Heavy boulder clay, grey in colour, tending to be wet, especially during winter.	4·5	12·7	3·8	21
	4·2	13·1	1·6	24
Herbage. Almost entirely *Nardus stricta*; frequently some *Vaccinium*.	5·2	15·3	6·9	17
	4·4	13·8	2·4	20
	5·4	13·3	10	27
	5·0	12·9	11	24
Type D				
Soil. Strongly acid peats occurring as thin layers (not more than 8 inches deep) on rocks (rhyolite, acid andesite and trachyte) with no underlying mineral soil. Different proportions of rock fragments produce the variations in loss on ignition.	3·3	70	22	43
	3·3	81	13	23
	3·3	84	8·5	22
	3·2	71	10	36
	3·1	78	2·8	39
	3·3	85	7·6	29
Herbage. Mainly *Calluna*; some *Vaccinium*; little or no grass.				
Peat from the Island of Lewis	4·7	83	29	22

The dry soils (Type B) mainly gave high results, especially in soil tests. The productivity of these soils is low and it is probable that under natural conditions their normal deficiency of moisture renders them unsuitable media for micro-organisms. Without mechanical treatment the soils would not become wet even after prolonged standing in contact with water. For the purposes of the soil tests it was necessary to mix thoroughly each sample with water by means of a spatula before adding the urea solution. The large quantity of readily available nutrient material contained in the broken-up, fresh rootlets in the samples probably also contributes to their urea-splitting activity under laboratory conditions. The results quoted in the table and also others which were

obtained with the same soil type did not indicate any relationship between the urea-decomposing power of the samples and their fertility as judged by the amount and quality of the herbage produced.

The *Nardus*-growing soils (Type C), which are representative of the least profitable grasslands, exhibited the smallest activity, but the tests in the soil itself demonstrated that they contained an active urea-decomposing microflora. After 48 hours' incubation the different samples had ammonified from 34 to 55 per cent. of the added urea.

The samples of peat from Boghall, which exhibited an extreme degree of acidity, produced only a slow decomposition in solution but gave surprisingly high results in the tests where urea was decomposed in the peat itself. In the latter tests the amount of dry peat used was reduced to 12·5 gm. in order to facilitate the determination of urea, but the usual quantity of urea was added. Thus, if the activity of the peat samples is to be compared with that of other soils on an equal dry weight basis the figures obtained in the "soil" tests with peat should be doubled. Peat from Lewis, which was less acid than the Boghall material, showed no greater activity in the decomposition tests.

THE DECOMPOSITION OF UREA IN AGRICULTURAL AND FOREST SOILS.

The urea-decomposing power of a number of soils occurring on different geological formations in the south-east of Scotland has been determined. The samples were taken from locations where cultivated land, permanent pasture and woodland adjoined each other. The three types were usually sampled at points not more than 50 yards apart. In this way the effect of cultivation and cropping of virgin land on the decomposition of urea could be studied. In choosing the locations for sampling an endeavour was made to avoid situations where the land had been allowed to grow timber, or had been converted into permanent pasture because the soil was less fertile than on the adjacent cultivated ground. The arable soils were under rotational cropping. The pasture soils examined had not been cultivated for at least 50 years. In sampling the forest soils the loose litter of undecomposed materials was first removed from the surface and the sample was taken from the next 2 inches, which generally included various proportions of a peaty layer.

The average results of the decomposition tests are shown in Table II, along with short notes on the soil types and the values for *p*H and loss on ignition of the samples used. As the three soils in each series were probably in all cases originally similar, the differences of loss on ignition between the arable, pasture and forest samples may be taken to represent

differences of organic matter content. Most of the pasture soils produced a very rapid decomposition of the urea. In both solution and soil tests they all showed greater activity than the samples from adjacent arable ground, and especially in soil tests the differences were frequently large. It is probable that the greater activity of pasture as compared with arable soils is exaggerated in laboratory experiments. Old pasture soils always contain an abundance of fibrous roots which are broken up when

Table II. *The decomposition of urea in various soils. Comparison of arable, pasture and forest soils.*

Soil type*, geological formation and locality	Series	Arable				Pasture				Forest			
				Decomposition (%)				Decomposition (%)				Decomposition (%)	
		*p*H	Loss on ignition	In solution	In soil	*p*H	Loss on ignition	In solution	In soil	*p*H	Loss on ignition	In solution	In soil
Blown sand; at margin of cultivation near the coast; from Dirleton, East Lothian	1	7·5	4·6	53	13	6·7	5·3	84	58	4·5	29·9	76	66
	2	5·6	5·1	11	2·3	7·4	6·0	52	31	6·2	11·3	60	20
Heavy clay; alluvium; from Dunmore Park and Grangemouth, Carse of Stirling	3	6·2	9·2	60	17	6·0	16·4	90	100	5·5	15·6	65	78
	4	6·0	9·7	33	7·8	5·8	14·0	90	98	5·8	12·2	69	50
Brown loam; derived from Lower Silurian; from Stobshiel, East Lothian	5	6·0	8·7	54	20	5·9	10·8	75	78	4·4	18·1	56	28
	6	6·3	8·0	58	26	5·8	12·7	77	97	4·2	38·0	26	30
Brown loam; glacial drift derived mainly from carboniferous limestone and calciferous sandstone; from East Saltoun, East Lothian	7	7·1	4·7	35	7·3	7·0	7·6	59	15	4·8	9·8	15	21
	8	7·5	6·5	39	11	5·4	10·4	71	97	4·4	15·1	16	13
Brown loam; glacial drift over trachyte; from Camptoun, East Lothian	9	7·4	7·6	69	12	6·9	9·4	85	88	5·7	9·7	46	18
	10	7·4	6·8	64	6·8	6·1	8·8	76	99	6·2	13·6	84	76

* The descriptions of the soils apply to the samples taken from the arable and pasture ground. The characteristics of the forest soils were in most cases considerably modified by accumulated organic matter.

the soil is sieved, thus providing the micro-organisms with an amount of fresh organic matter which would only become slowly available to them in an undisturbed soil. It has been shown(9) that additions of fresh organic matter to soils enable the micro-organisms to decompose urea more rapidly. Two pasture samples were much less active than the others in both tests: these were the most alkaline samples and they both gave a comparatively low loss on ignition. Some of the forest soils were decidedly more active in one or both of the tests than samples of neighbouring arable soils, but the differences between the two types

were variable and in certain cases were not significant. The woodland samples which showed the least activity gave either a low *p*H value or a low loss on ignition. Some of the forest samples examined approach closely to peat in their characteristics.

THE DECOMPOSITION OF UREA IN ALKALINE SOILS.

Tests were made with a few alkaline soils in order to widen the survey of soil conditions. Short descriptions of the soils used and the results of the decomposition tests are given in Table III.

Table III. *The decomposition of urea in alkaline soils.*

Soil type, geological formation and locality	*p*H	Loss on ignition	Urea decomposed (%) In solution	In soil	
Brown loam containing many fragments of limestone; glacial drift over carboniferous limestone; from East Saltoun, East Lothian	7·5	15·4	82	1 day	13
Clay; under irrigation; alluvium; from Gemmeza, Gharbia, Egypt	8·0	9·6	47	1 day	11
				7 days	37
				14 days	62
Tenacious, sticky clay; unfertile; alluvium; from Sidi Omar, Edfina, Egypt	8·7	9·9	6·9	1 day	0
				7 days	3·0
				14 days	5·2
Tenacious, sticky clay; unreclaimed alkali soil; alluvium; from Delengat, Egypt	9·0	6·7	6·4	1 day	3·6
				7 days	18
				14 days	30

The two most alkaline samples gave low results but in solution tests both produced an active decomposition after the second day of incubation. It is probable that factors other than alkalinity are responsible for reducing biological activity in the sample from Edfina in Egypt.

DISCUSSION.

There has been much controversy concerning the relative merits of soil and solution tests carried out in the laboratory for the purpose of measuring the activities of micro-organisms in soils. Both tests were used in this work as each appeared to be capable of yielding valuable information. There has, however, been no general agreement between the results obtained by the two methods. It will be observed from the tables that the arable soils gave a greater percentage decomposition of urea in solution than in soil tests while the situation is the reverse with all except two of the hill and heath samples. The conditions under which the decomposition is carried out in the soil tests probably approach

most nearly to those in the field; but such tests are also distinctly artificial, especially in the case of marshy or permanently dry soils. It would appear that solution tests measure chiefly the activity of bacteria, while decomposition experiments in soil give information concerning the soil conditions and possibly also the activity of fungi.

The experiments described above were made without regard to the season of the year, a factor which may have modified the results to some extent. A seasonal variation in the activity of the soil micro-organisms which decompose urea has been found by means of solution tests (21, 24), but attempts to demonstrate this phenomenon by employing either solution or soil tests with a garden soil in the neighbourhood of Edinburgh have failed. The results presented in this paper were obtained with samples collected during the periods January to May and August to September.

The chief result of this investigation is that urea is decomposed with extraordinary rapidity in many soils, and no soil of agricultural value has been encountered in which the decomposition took place slowly as measured by the standards of other microbiological processes. A sufficient number of soil types, including extremely acid peat and strongly alkaline soils, have been examined to justify the assumption that urea may be decomposed readily in all soils capable of supporting plant growth. The rate at which urea may be ammonified is probably greater under all soil conditions than that of nitrification. Although urea is easily washed out of soils, the rapidity with which it is converted to ammonia will largely preclude a loss of nitrogen in this way. It is unlikely that inactivity of urea-decomposing micro-organisms is responsible for a slow availability of calcium cyanamide in any soil.

In view of the facility with which micro-organisms decompose urea the soil conditions which favour or retard their action are of minor importance. The number of samples of each soil type examined in this work was small, while the number of types was considerable, and a detailed analysis of the soil factors influencing the decomposition is impossible. A few tentative conclusions can, however, be drawn from the results. The greater activity of pasture soils and many forest soils as compared with those from adjacent cultivated ground is most probably attributable to their greater content of organic matter. The same factor is probably responsible for the superiority in soil tests of most of the hill and heath samples to those of the arable land. The effect of soil reaction, although moderated or magnified by other factors, is discernible among the results. In soil tests the most active samples of

cultivated soil had *p*H values between 6·0 and 6·5, while the most active of the pasture soils were more acid (*p*H 5·4 to 6·1). Activity tended to decrease in the more acid samples and, to a still greater degree, in alkaline soils. In solution tests, however, the acid soils tended to give low results while the alkaline samples were relatively more active than in soil tests.

It might have been expected that the ammonification of urea would take place most rapidly in soils to which farmyard manure or animal urine were frequently added. The experiments described above, however, give little indication of such a relationship. The soils of woodlands which are seldom frequented by animals may be capable of producing a very active decomposition of urea.

SUMMARY.

The decomposition of urea has been investigated in 59 soil samples of extremely varied character. Urea was decomposed readily in all and very rapidly in most of the samples.

In mountain and heath soils the decomposition was generally active, especially in soil tests. Strongly acid peat samples (*p*H 3·1 to 3·3) decomposed from 0·44 to 0·86 per cent. of their own dry weights of urea in 24 hours at 22° to 23° C.

In solution and soil tests samples from permanent pastures produced a more rapid decomposition than those from cultivated land. In soil tests with pasture samples quantities of urea as large as 1 per cent. of the dry weight of soil were converted to ammonia in 24 hours at 22° to 23° C. Fertile arable soils produced a relatively slow decomposition in soil tests.

Samples of forest soils were generally more active than those from cultivated ground.

Strongly alkaline soils showed comparatively little activity in soil tests.

Although urea is not absorbed by soils its rapid conversion to ammonia will generally prevent losses by leaching.

I would like to express my thanks to Dr W. G. Ogg for his assistance in the selection and classification of the soils which were used in this work.

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(*Received May 23rd*, 1930.)

THE AVAILABILITY OF PHOSPHATIC FERTILISERS AS SHOWN BY AN EXAMINATION OF THE SOIL SOLUTION AND OF PLANT GROWTH[1].

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(With Two Text-figures.)

Introduction.

Recent work in this country (1, 2, 3, 4), including an earlier paper by the writer, and in America by Burd and Martin (5), has indicated that for any given uncropped soil the concentration of phosphate in the soil solution remains at a more or less constant value, no matter what phosphatic manure the soil receives, if any. If this result is definitely established, then it is evident that the relative efficiencies of different phosphatic manures must be conditioned by factors other than the actual concentration of phosphate in the soil solution of the uncropped soil. When the soil is cropped, however, absorption of phosphate by the roots of the growing plant must tend naturally to lower the phosphate concentration of the soil solution, and it would seem possible that the relative efficiencies of different phosphatic manures may be related to their relative rates of solution, that is to say the speed with which they can re-establish the phosphate concentration of the soil solution, in the face of agencies which tend to reduce this concentration.

An attempt has been made accordingly, in the investigation reported, to study for a given soil the effect of various treatments on both the concentration of phosphate in the soil solution and the growth of the plant.

Objects and outline of investigation.

Two pot-culture trials were carried out, the first in 1925 and the second in 1926, on a medium loam soil, belonging to the millstone grit formation, from the Stalybridge district of Cheshire. The soil was deficient in phosphate and had a lime requirement of 5 tons calcium car-

[1] (Being an abridged form of a Thesis approved for the Degree of Doctor of Philosophy in the University of London.)

bonate per acre. The investigation was carried out in parallel on the soil limed with Billingham carbonate of lime at the rate of (*a*) half, and (*b*) twice the lime requirement.

The first trial consisted of a study of the simultaneous effects of the addition of phosphatic fertilisers to the limed soils, on plant growth, and on the concentration of phosphate in the soil solution of the soil both cropped and uncropped, whilst in the second trial the separate and combined effects on plant growth of the addition of phosphatic fertilisers to the soils, the time of application of the lime and phosphatic fertilisers and the date of sowing the seed, were studied. Two phosphatic treatments, a low-grade, high-soluble slag, and superphosphate, applied at the rate of 50 lb. P_2O_5 per acre, together with a control, were employed throughout, whilst barley was the crop grown. In all cases adequate dressings of potash and nitrogen were given.

I. 1925 TRIAL.

Description.

The soils were limed in bulk early in April, and the pots, 8 in. waxed, fire-clay pots, filled at the end of the month, the phosphates being applied at the same time; the potash and a first application of nitrogen were added in solution a day or two after, whilst a further application of nitrogen was given at the time of tillering. The barley was sown on May 4 and the plants thinned out later to one per pot. The pots were watered as necessary, keeping the soil moisture contents in all cases as nearly as possible equal, so far as could be judged by observation.

Samples were taken from each treatment periodically over the growing season and the concentration of phosphate in the soil solution, and the plant dry weight, determined.

Methods of sampling and analysis.

In the case of the cropped treatments, growth measurements were taken for each plant and the averages for the treatment calculated. The plants were then divided into four groups, in order of the measurements, and one plant selected from each group, such that the average measurements of the selected plants corresponded with those for the treatment. From the uncropped series two pots of each treatment were selected each time.

In all cases, to avoid any immediate disturbance of the soil solution by watering or by rains, the selected pots were kept under cover for a day before examination and were not watered during this time.

The soil solution was obtained by Parker's displacement method (6), using ethyl alcohol as the displacing liquid and the phosphoric acid in the solution was determined, after removal of organic matter and silica, by Kleinmann's nephelometric method (7). In this method the phos-

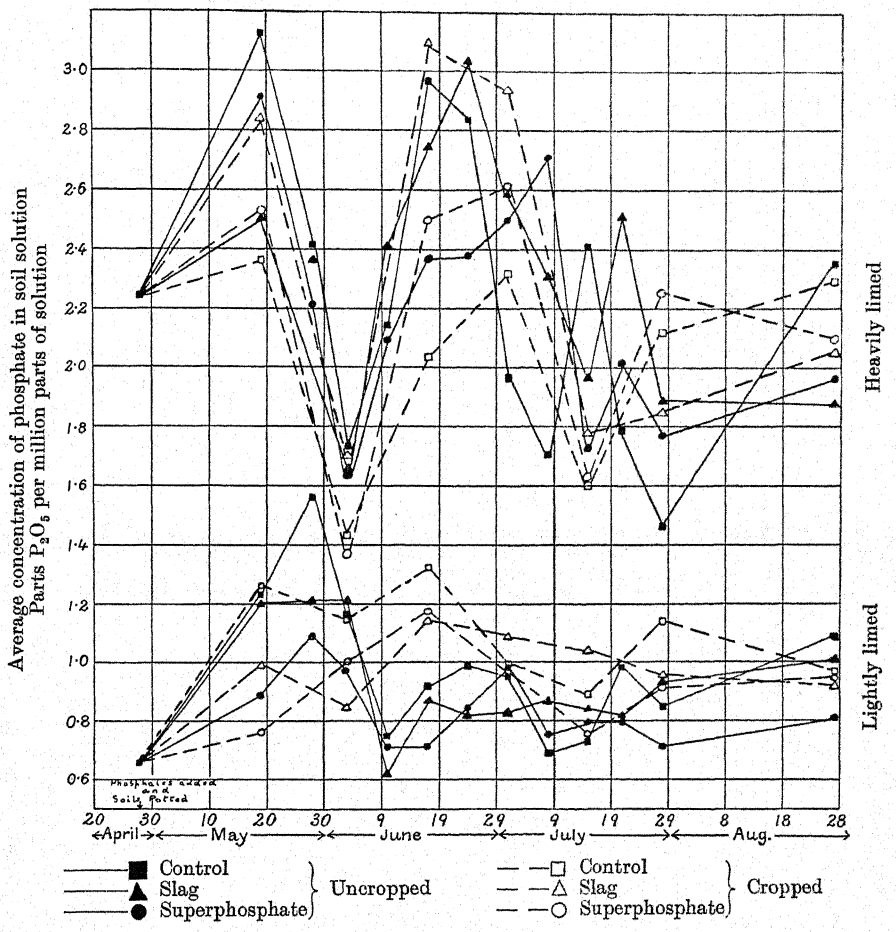


Fig. 1. Curves showing fluctuations in average concentrations of phosphate in soil solution. 1925 trial.

phoric acid in 25 c.c. solution, containing from 0·0005 to 0·1 mgm. P_2O_5, is precipitated by means of a specially prepared strychnine-molybdic acid reagent, and the turbidity produced compared nephelometrically with that of a standard phosphate solution of approximately the same concentration, treated similarly.

The analytical procedure was as follows:

A suitable volume of soil solution was evaporated to dryness after the addition of a few drops of concentrated hydrochloric acid. The residue was ignited gently to dehydrate the silica and to char thoroughly the organic matter and then cooled. 2 c.c. 7*N* HCl (sp. gr. 1·115) were added, the residue moistened and left one minute and about 20 c.c. hot water added. The solution was kept warm for 15 to 20 minutes and the silica and carbonaceous matter filtered off and washed with hot water. The solution was evaporated down, transferred to a stoppered cylinder, made up with water to 23 c.c. and the phosphate precipitated with 2 c.c. of the strychnine-molybdic acid reagent.

The method, when tested with artificial soil solutions containing from 0·01 to 0·10 mgm. P_2O_5, gave a maximum analytical error of 1·25 per cent.

Results.

The average values for the concentration of phosphate in the soil solution, under the various treatments, during the period of the trial, are shown graphically in Fig. 1, and the average seasonal concentrations are given in Table I.

Table I. *Average seasonal concentrations of phosphate in soil solution (p.p.m. P_2O_5).* 1925 *trial.*

Soil treatment	Control		Slag		Superphosphate	
	Uncropped	Cropped	Uncropped	Cropped	Uncropped	Cropped
Lightly limed	0·99	1·10	0·94	1·00	0·84	0·93
Heavily limed	2·24	2·02	2·32	2·32	2·19	2·13

Table II. *Average final yields of straw and grain, and maximum tiller numbers.* 1925 *trial.*

Soil treatment	Straw Dry weight (gm. per plant)	Grain		Maximum tiller number per plant
		Dry weight (gm. per plant)	1000-corn dry weight (gm.)	
Lightly limed: Control	2·283	1·619	22·18	7
Slag	2·398	1·676	22·35	6
Superphosphate	2·745	3·148	27·86	7
Heavily limed: Control	5·078	2·581	19·26	14
Slag	6·242	4·143	24·96	15
Superphosphate	5·418	4·129	27·52	12

The average plant dry weights at successive stages of growth, for the various treatments, are shown graphically in Fig. 2, and the average

final yields of straw and grain, and the maximum tiller numbers, are given in Table II.

Throughout the trial the figures for individual pots of a sample were generally in good agreement and the average values given may be regarded therefore as representative.

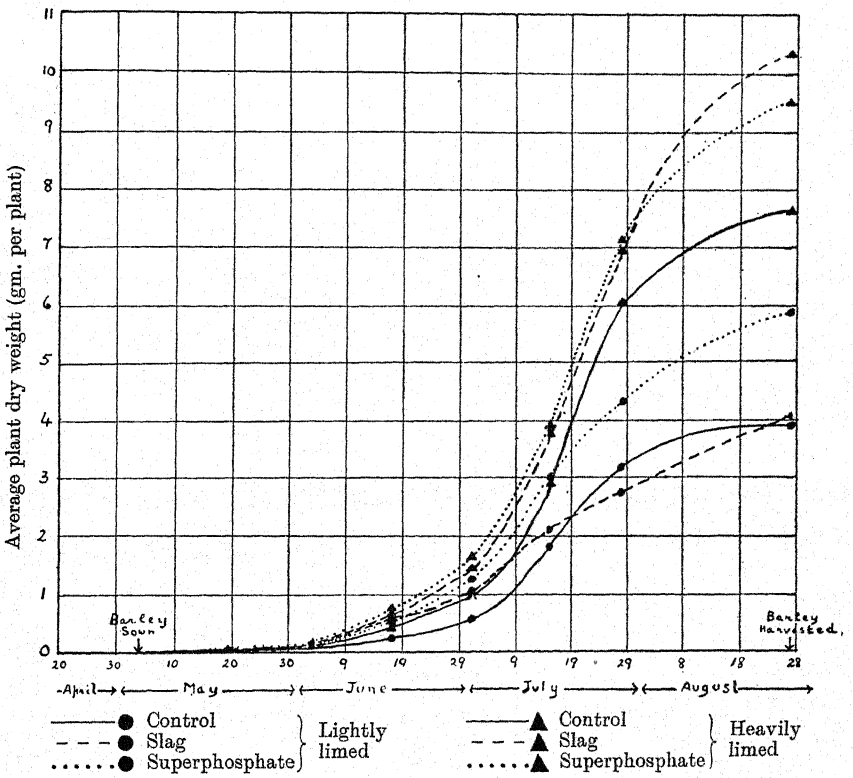


Fig. 2. Curves showing average plant dry weights at successive stages of growth. 1925 trial.

Discussion of results.

The light dressing of lime increased the phosphate concentration of the soil solution from 0·66 p.p.m. P_2O_5 in the untreated soil to about 1 p.p.m., whilst the heavy dressing increased the concentration to over 2 p.p.m. P_2O_5. The application of phosphates to the limed soils and cropping the soils affected the concentration to a much smaller extent, though the changes were in most cases significant.

The concentration in the untreated soil was practically constant. After treatment, particularly on the heavily limed soils, the concentration

fluctuated considerably, though it tended to become more constant later. A striking feature of the fluctuations was that their general trend was similar for the control and phosphate treatments alike and on the heavily limed soils similar on both the cropped and uncropped soils; further, more particularly on the uncropped soils, the fluctuations were lesser in degree on the phosphate than on the control treatments. It was thought that the fluctuations might be due to variations in the soil moisture content, soil temperature or climatic factors, but no evidence of this nature could be found and the conclusion must be drawn that the fluctuations were one of the effects of liming; the reason for the influence of the application of phosphates on the fluctuations is, however, not apparent.

The heavy dressing of lime, compared with the light dressing, increased the growth of the crop as shown by the number of tillers developed and the yields of straw and grain; it had no appreciable effect, however, on the 1000-corn weights. The application of phosphates to the limed soils had no effect on tillering but markedly increased the yield of grain and the 1000-corn weight; the yield of straw was increased to only a small extent. Slag was as effective as superphosphate on the heavily limed soil but less effective on the lightly limed[1]. The yields call for little comment, except that the poor results obtained with slag on the lightly limed soil after the middle of the season cannot readily be accounted for. In a series of small plots laid out *in situ* on the soil in Cheshire and manured and cropped similarly, it is significant to note that the plot yields at harvest were relatively in close agreement with those obtained in the pots, except that on the lightly limed soil the slag and superphosphate treatments did equally well.

The effects on the concentration of phosphate in the soil solution, of the application of phosphates to the limed soils and of cropping the soils, were in general rather surprising and with our present knowledge of the complex conditions in the soil governing the phosphate concentration of the soil solution, difficult to explain. The application of phosphates in the case of the lightly limed treatments, cropped and uncropped, depressed the concentration, and on the heavily limed treatments had variable effects; at the same time the superphosphate treated soils showed usually rather lower concentrations than the slag treated. The generally accepted reactions which take place when soluble phosphates are added to soils, would explain of course why there may be no

[1] The lightly limed slag treated plants made as good growth as the corresponding superphosphate plants up till mid-season, after which the former deteriorated markedly.

permanent *increase*, but they do not explain why there should be a decrease in the concentration. Cropping would be expected, if the plant obtains its phosphate from the soil solution, as is generally accepted, to reduce the phosphate concentration of the solution, and it would seem possible that the relative efficiencies of different phosphatic manures may be related to the speed with which they can re-establish the concentration, in the face of absorption of phosphate from the solution by the plant. Only in the case of the heavily limed control soil, however, did cropping reduce the concentration appreciably; on the heavily limed phosphate treated soils the effect was variable, whilst on each of the lightly limed soils cropping actually increased the general concentration to a significant extent. Further, no apparent relationships can be traced between the concentration of phosphate in the soil solution under the various soil treatments and the corresponding crop yields at successive stages of growth.

It would appear, therefore, that the relative availabilities of different phosphates, as measured by crop growth, are not related to the concentration of phosphate in the soil solution (except in so far as the concentration is dependent on the lime status of the soil) nor to the ability of the phosphate to maintain the concentration in the face of absorption of phosphate from the solution by the roots of the growing plant. The growth of the plant and hence, presumably, the supply of phosphate to the plant, were apparently governed by two factors, an adequate lime status and the presence in the soil of sufficient phosphate in a form suitable to be taken up by the plant; the varying availabilities of different phosphates to a particular crop would appear to be due, therefore, to differences in the chemical nature of the phosphates themselves, operating otherwise than through their solubilities. Further, the general lack of correlation between the concentration of phosphate in the soil solution and the growth of the crop, suggests a distinct possibility of the plant roots taking up phosphate direct, probably through contact with the soil colloids, rather than from the soil solution. This theory has been put forward in the past both by Comber (8) and Ramann (9), but has had little evidence to support it. The theory would account, however, for the unexpected results obtained in the trial, and at the same time explain the general conclusions arrived at above.

Simultaneously with the work carried out by the writer and unknown to him at the time, investigations of a somewhat similar nature were carried out in America by Parker and Tidmore (10) and in Germany by von Wrangell and her co-workers (11, 12, 13, 14); in both cases the results

were in close agreement with those obtained by the writer, except that von Wrangell expressed the soil solution and found its phosphate concentration to be a function of the moisture content of the soil. In discussing their results, the authors suggest, as Marais (15) had done previously, that the concentration of phosphate in the soil solution at the surface of a soil particle is greater than that in the bulk of the solution, and that consequently plants may take up their phosphorus at the surfaces of soil particles from a more concentrated solution than that obtained by displacement or expression. While it is possible, of course, that some phosphate may be taken up in this way, a more likely explanation of the facts would seem to be that the roots make actual contact with the soil particles and take up phosphate direct; further evidence of this may be cited in that the root hairs of plants are often found not merely in loose contact with particles of soil, but actually united with the soil and inseparable from it without breaking the root.

II. 1926 TRIAL.

Description.

Since the yields as a whole in the 1925 trial were somewhat low, and this was thought to be due probably to the rather late sowing of the barley, it was decided to examine in 1926 the effect on the yields of different dates of sowing. At the same time the soils used in 1925 were reserved[1] and the residual effects of the lime and phosphates investigated, and the yields compared with those obtained on soil freshly and similarly treated. The 1925 soils received no further applications of lime, potash or phosphate, but further applications of nitrogen were given as for the freshly treated soils. The latter were limed in bulk early in February, and the phosphates, potash and nitrogen added as in the previous season.

The trial was divided into three Sets, A, B and C, according to the date of sowing, and the pots, with one or two unavoidable exceptions in Set C, run in triplicate. Three plants were grown per pot, 11 in. waxed pots being used; the soils were potted in all cases the day before the barley was sown. The pots were watered as in 1925.

The barley was sown in the three sets respectively on March 18, April 8 and April 28, the corresponding dates of harvesting being August 19, August 28 and September 16.

[1] Only soils from the uncropped pots and those cropped pots sampled before the plants had made any appreciable growth, and not used for displacement of the soil solution, were reserved.

Results.

The yields of individual pots were generally in good agreement, and the average straw and grain yields are given in Tables III and IV. The average 1000-corn weights are given in Table V and the maximum tiller numbers in Table VI.

Table III. *Average yields of straw (gm. dry matter per pot).* 1926 *trial.*

		Soil treatment					
		Lightly limed			Heavily limed		
Set	Time of soil treatment	Control	Slag	Super-phosphate	Control	Slag	Super-phosphate
A	1925	14·93	17·43	17·13	22·80	24·30	23·71
	1926	16·24	22·30	20·31	16·68	22·71	22·61
B	1925	10·55	11·70	11·11	16·70	21·71	20·86
	1926	10·75	13·81	11·62	14·57	20·29	17·66
C	1925	9·11	11·71	—	17·37	18·84	—
	1926	9·94	11·76	10·42	10·94	15·96	15·33

Table IV. *Average yields of grain (gm. dry matter per pot).* 1926 *trial.*

		Soil treatment					
		Lightly limed			Heavily limed		
Set	Time of soil treatment	Control	Slag	Super-phosphate	Control	Slag	Super-phosphate
A	1925	10·63	12·15	10·10	20·21	22·47	22·65
	1926	11·47	21·36	23·76	9·38	21·15	20·40
B	1925	5·52	7·96	7·51	15·66	19·52	17·57
	1926	8·33	14·32	13·38	11·73	18·61	15·99
C	1925	3·90	6·94	—	11·77	11·94	—
	1926	4·18	8·83	9·51	5·03	9·69	11·18

Table V. *Average 1000-corn weights (gm. dry matter).* 1926 *trial.*

		Soil treatment					
		Lightly limed			Heavily limed		
Set	Time of soil treatment	Control	Slag	Super-phosphate	Control	Slag	Super-phosphate
A	1925	49·12	42·23	43·37	39·54	36·34	38·10
	1926	42·50	50·70	44·25	44·73	39·44	37·70
B	1925	38·20	38·11	43·44	34·24	38·43	37·90
	1926	41·10	43·50	45·80	43·20	36·52	34·27
C	1925	26·75	28·70	—	32·60	32·20	—
	1926	27·85	37·14	38·25	44·90	38·92	38·35

Table VI. *Maximum tiller numbers. Average per plant.* 1926 *trial.*

Set	Time of soil treatment	Soil treatment: Lightly limed: Control	Lightly limed: Slag	Lightly limed: Superphosphate	Heavily limed: Control	Heavily limed: Slag	Heavily limed: Superphosphate
A	1925	2	2	3	7	9	10
	1926	3	7	6	3	8	8
B	1925	1	1	1	11	10	9
	1926	3	8	6	5	12	10
C	1925	0	1	—	10	10	—
	1926	4	7	5	4	9	9

Discussion of results.

The main features of the results may be stated briefly as follows:

1. The application of phosphates resulted in increases in yields which were greater on the freshly treated soils. Tillering was improved also in the latter cases. Where the soils were treated the previous year the effect was mainly on the grain, and there was no increase in tillering. Slag and superphosphate behaved similarly throughout.

2. The lightly limed soils, particularly the phosphate treated, produced more extensive tillering and higher yields where the soils were freshly treated than where treated the previous year. On the heavily limed soils an opposite effect was observed, and the differences were more marked on the control than on the phosphate treatments.

3. On the soils treated the previous year, the heavily limed treatments produced more extensive tillering and higher yields than the corresponding lightly limed treatments. On the freshly treated soils similar effects were observed with the later sowings but with the early sowing corresponding lightly and heavily limed treatments gave almost identical results.

4. With successive sowings the yields were markedly decreased, but the extent of tillering was not appreciably affected.

5. The 1000-corn weights varied in a less definite manner, but were generally higher on the lightly than on the heavily limed treatments. Lateness of sowing adversely affected the weights on the former, though not on the latter treatments.

Two important points emerge from the results, firstly the influence of the time of application of the lime and phosphates on their effectiveness, and secondly the influence of the date of sowing the barley on the growth of the crop and to some extent on the effectiveness of the lime and phosphates.

The light dressing of lime alone was slightly more effective in the year of application than a year later, or in other words, had become less effective after being in the soil a year. Now the lime used was a quick-acting form, and it is probable that on such an acid soil the light dressing had been used up completely the year previously, and that consequently no free lime was left in the soil to exert any effect in the second year. Where phosphates were applied with the light dressing of lime, the superiority of the fresh over the previous year's application was much more marked.

It is probable that the heavy dressing of lime performed two separate functions in the soil, firstly the correction of acidity, and secondly the rendering available to the plant the original reserves of phosphate and other plant nutrients present in the soil. The first function was found in 1925 to be achieved very rapidly, but the second would appear to be of longer duration, the results being much more apparent a year after than in the season immediately following liming. Thus to obtain the best results immediately after adequate liming of an acid soil deficient in available phosphate, the addition of phosphate in a soluble form will be necessary; but by the following year the lime will have rendered much of the phosphate originally present in the soil available, and that applied with the lime will consequently not show to the same advantage in the second year as in the year of application, though its effects may be still, to some extent, apparent.

It has, of course, long been known that early sowing is essential in the case of many crops, including barley, to obtain high yields of good quality produce, but the actual extent to which the yields and quality may be affected by the date of sowing is very striking and probably not always fully realised. The date of sowing influenced also to some extent the effectiveness of manures applied. In particular the effectiveness of the phosphates became generally less marked on the lightly limed soils with the later sowings, whilst the relative effects of the light and heavy dressings of lime in the case of the freshly treated soils were also considerably influenced by the date of sowing.

Other interesting features of the results are the peculiar variations in the 1000-corn weights, the striking similarity under the many different conditions between the two phosphates used, and the comparative effects of the various factors on the straw and grain yields, the grain being affected in general to a greater extent than the straw yields.

Finally, a comparison of the yields obtained in 1925 with those obtained under the same treatments in 1926 is given in Table VII. On

the lightly limed treatments higher yields of both straw and grain were obtained in 1926 than in 1925, the differences being particularly marked in the case of the slag treatment, where no "falling off" was apparent at mid-season in 1926 as was the case in 1925. On the heavily limed treatments, the yields of grain were in each case practically the same in the two seasons, but the yields of straw were lower in 1926 than in 1925, particularly on the control and slag treatments. The 1000-corn weights were much higher in 1926 than in 1925, and varied in different manners in the two seasons.

Table VII. *Comparison of yields of straw and grain.* 1925 *and* 1926 *trials*[1].

	Straw		Grain			
	Average dry weight (gm. per plant)		Average dry weight (gm. per plant)		1000-corn dry weight (gm.)	
Soil treatment / Season...	1925	1926	1925	1926	1925	1926
Lightly limed: Control	2·28	3·31	1·62	2·50	22·18	27·86
Slag	2·40	3·92	1·68	3·41	22·35	37·14
Superphosphate	2·75	3·47	3·15	3·73	27·86	38·25
Heavily limed: Control	5·08	3·65	2·58	2·41	19·26	44·90
Slag	6·24	5·32	4·14	3·70	24·96	38·92
Superphosphate	5·42	5·11	4·13	4·41	27·52	38·35

[1] Soils freshly limed and phosphates applied at time of sowing in each case; barley sown on May 4 in 1925 and on April 28 in 1926.

Summary.

The growth of barley and the phosphate concentration of the soil solution, of the soil cropped and uncropped, were examined simultaneously in pot cultures, after treatment of an acid soil deficient in phosphate, with lime with and without slag or superphosphate.

Liming at the rate of half the lime requirement increased the concentration from about 0·7 p.p.m. P_2O_5 to about 1 p.p.m. and at the rate of twice the lime requirement to over 2 p.p.m. P_2O_5. Phosphates depressed the concentration on the lightly limed soils, and on the heavily limed had variable effects. The superphosphate-treated soils showed rather lower concentrations than the slag-treated. Cropping raised the concentration on the lightly limed treatments, but on the heavily limed reduced it on the control and had a variable effect on the phosphate treatments.

The concentration in the untreated soil was practically constant; after treatment the concentration fluctuated considerably, but tended to

become more constant later. No relationship could be traced between the concentration and the soil-moisture content.

The heavily compared with the lightly limed treatments produced more extensive tillering and higher yields. Phosphates increased the yields, particularly of grain, but had no effect on tillering. Slag and superphosphate were equally effective on the heavily limed soil, but superphosphate was the more effective on the lightly limed.

No correlation between crop growth and the concentration of phosphate in the soil solution was apparent. The possibility of plant roots taking up phosphate direct is suggested.

In further pot cultures, studying the influence on crop growth of the time of application of the lime and phosphates and of the date of sowing the barley, higher yields were obtained from freshly treated soils than from soils treated a year before sowing on the lightly limed treatments, whilst the reverse was the case with the heavily limed.

On the soils treated the previous year the heavily limed treatments gave higher yields than the lightly limed; on the freshly treated soils the two gave similar yields with early sowing, but the heavily limed gave the higher yields with later sowings.

Slag and superphosphate gave similar results throughout, and their application was more effective on the freshly treated soils than on those treated the previous year.

The yields were reduced markedly in three successive sowings.

ACKNOWLEDGMENT.

The work reported was undertaken during the tenure of a Ministry of Agriculture Research Scholarship in Soil Chemistry, the 1925 trial being carried out at the Rothamsted Experimental Station and that of 1926 at the Woburn Experimental Farm Pot Culture Station. For these privileges the writer thanks Sir John Russell, Director of Rothamsted and Dr J. A. Voelcker, Director of Woburn. Thanks are tended also to Mr H. J. Page, formerly Head of the Chemistry Department at Rothamsted, under whose helpful supervision the work was carried out.

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(*Received July 14th*, 1930.)

INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

By the Agricultural Research Staff of Imperial Chemical Industries, Limited.

I. THE CHEMICAL COMPOSITION OF INTENSIVELY TREATED PASTURE.

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(With Four Text-figures.)

Introduction.

The investigations reported were undertaken to gain exact information concerning the chemical composition of pasture as grazed under the Intensive System of Grassland Management; the work forms part of a scheme of research being conducted at this station, in which the various aspects of the Intensive System are being studied.

The Intensive System, which had its origin in the work of Dr Warmbold at Hohenheim in Germany during the War, was introduced into this country in 1926, and consists in the production throughout the grazing season, of a continuous supply of young herbage, this being achieved by the rotational grazing of the pasture, which receives adequate dressings of lime, phosphoric acid and potash, together with successive and rotational dressings of nitrogen over the late winter, spring and summer months.

The treatment and management of pastures under the Intensive System are, of course, departures from past agricultural practice, and their effects on the chemical composition of the herbage were completely unknown at the time of the introduction of the Intensive System; in fact, apart from the work of Woodman and his associates(1) at the Cambridge University School of Agriculture the previous year, practically no exact data on the nutritive value of young grass were available.

Woodman, in 1925, showed that the dry matter of pasture cut at weekly intervals, was a concentrated feeding stuff, rich in digestible protein and low in fibre and, further, that the nutritive value was but

little diminished during mid-season. These results, however, were obtained on unmanured pasture which was not actually grazed.

With the introduction of the Intensive System of Grassland Management, it was decided to investigate the chemical composition of pasture as produced and grazed under that system, that is to say, pasture intensively manured and rotationally grazed at a stage of growth which ranges usually between three and five weeks.

Outline of work.

The following investigations were carried out over the period 1926 to 1929:

I. 1926. A chemical examination was made of a number of samples of pasture as grazed during the season, on the Intensive System paddocks at Tollesby Farm, Marton, North Yorkshire. The work was of a preliminary nature.

II. 1927. In 1927 a systematic study of the chemical composition of intensive and non-intensive pastures was made at Tollesby Farm, and of intensive pastures at a second centre, Melchet Court, Romsey, Hampshire; samples of the herbage from each paddock were examined at each grazing throughout the season, when the first-line stock were about to be turned on to the paddock.

III. 1928. The work this year referred mainly to the productivity of pastures farmed under the Intensive System.

IV. 1929. A systematic study was made in 1929 of the chemical composition and productivity of the pastures under the Intensive System of Management at the farm of the Imperial Chemical Industries, Limited, Agricultural Research Station, Jealott's Hill, Berkshire. Samples were taken from each paddock each time that the first-line stock were about to be turned on; the herbage of a number of random quadrats, each 1 square yard, was cut close to the ground, weighed and a representative sample examined chemically.

In this paper are reported the chemical composition data obtained in 1926, 1927 and 1929 with respect to the organic constituents: protein, ether extract, fibre and nitrogen-free extractives. Mineral data were obtained also in 1927 and 1929; it is proposed to publish the results separately in a subsequent paper. The productivity data of 1928 and 1929 will also be dealt with in a later paper.

DESCRIPTION OF CENTRES.

(i) *Tollesby Farm, Marton, North Yorkshire.*

Twenty-seven acres of permanent pasture, varying from medium to rather poor in quality and divided into six paddocks, were farmed under the Intensive System in 1926, whilst in 1927 were added 12 acres of good permanent pasture, divided into three paddocks. The land is medium to somewhat heavy loam.

The pastures had received recently only basic slag, and the land had a high lime deficiency. Eight of the paddocks were treated at the commencement with lime, phosphoric acid and potash, followed by successive dressings of nitrogen, whilst one of the three added in 1927 was retained as a non-intensive paddock and received only phosphoric acid; this paddock was grazed rotationally, however, with the others. The six 1926 paddocks received phosphoric acid and potash also in 1927. The intensive paddocks received an average of three dressings of nitrogen per season, sulphate of ammonia at the rate of 1 cwt. per acre being employed in most cases and nitro-chalk, nitrate of lime and urea at equivalent rates in the remainder.

(ii) *Melchet Court, Romsey, Hampshire.*

Following a small-scale trial in 1926, 30 acres of old park and recently broken-down arable land, varying from moderately good to very poor in quality, and divided into eight paddocks, were farmed under the Intensive System in 1927. The soil is heavy and impervious. The land had had no manuring for many years and was very acid; the paddocks received in 1927 a complete manuring similar to that given at the previous centre.

(iii) *Imperial Chemical Industries, Limited, Agricultural Research Station, Jealott's Hill, Berkshire.*

Leaks Meadow, a permanent pasture of 16½ acres on the Research Station Farm, divided into eight paddocks, has been farmed under the Intensive System since late 1927. The pasture previous to treatment was medium to poor, but showed no marked signs of acidity; the soil is a somewhat impervious heavy loam. The paddocks received phosphoric acid, potash and nitrogen in 1928, and lime, phosphoric acid and an average of three dressings of nitrogen in 1929.

Methods of sampling and analysis.

The method of sampling employed in 1926 and 1927 was to cross the paddock from one corner to the diagonally opposite corner, in zigzag fashion, touching the two opposite sides three times each. A sample of the herbage was taken at regular intervals throughout the path and the small samples mixed. A representative sample was oven-dried and examined chemically by standard methods of analysis. The procedure of taking the sample was to pluck the herbage close to the ground, taking care that no roots were actually pulled up, nor any foreign material included in the sample.

The method of sampling employed in 1929, the cutting of random small squares, has already been described (p. 574).

Weather and management conditions.

The three seasons 1926, 1927 and 1929 afford very marked contrasts. 1926 may be considered a normal season; in 1927 an early drought was followed by an exceptionally wet summer, whilst 1929 provided one of the driest seasons on record. The grazing conditions were very different, therefore, in the three years. The distribution of rainfall over the period March to September, in the three years, at the centres concerned, is shown in Table I, and a note on the particular conditions at the respective centres follows.

Table I. *Distribution of rainfall (in inches), March to September, 1926, 1927 and 1929.*

Year	Centre	March	April	May	June	July	Aug.	Sept.	Total over period
1926	Tollesby Farm	0·76	1·02	1·43	2·24	4·63	1·96	1·95	13·99
1927	Tollesby Farm	1·83	1·41	1·66	2·31	4·32	4·69	3·37	19·59
1927	Melchet Court	3·68	1·80	0·64	2·51	3·24	3·83	5·53	21·23
1929	Jealott's Hill	0·03	1·07	3·15	0·75	1·51	1·18	0·38	8·07

(i) 1926. *Tollesby Farm.*

Following a fine dry period in early spring, the rainfall was adequate and well distributed; there was, in consequence, a sufficiency of young grass for grazing purposes throughout the season. The paddocks were grazed by sheep during the latter half of March, and by the dairy herd from the beginning of April until the middle of October.

(ii) 1927. *Tollesby Farm.*

Following a normal March and April, cold winds and drought prevailed in May and June, whilst the summer months were exceptionally wet. The grass on the intensive paddocks during April and May was plentiful and of markedly leafy growth, whilst during June and the early part of July, growth was of a stunted nature and many short flowering

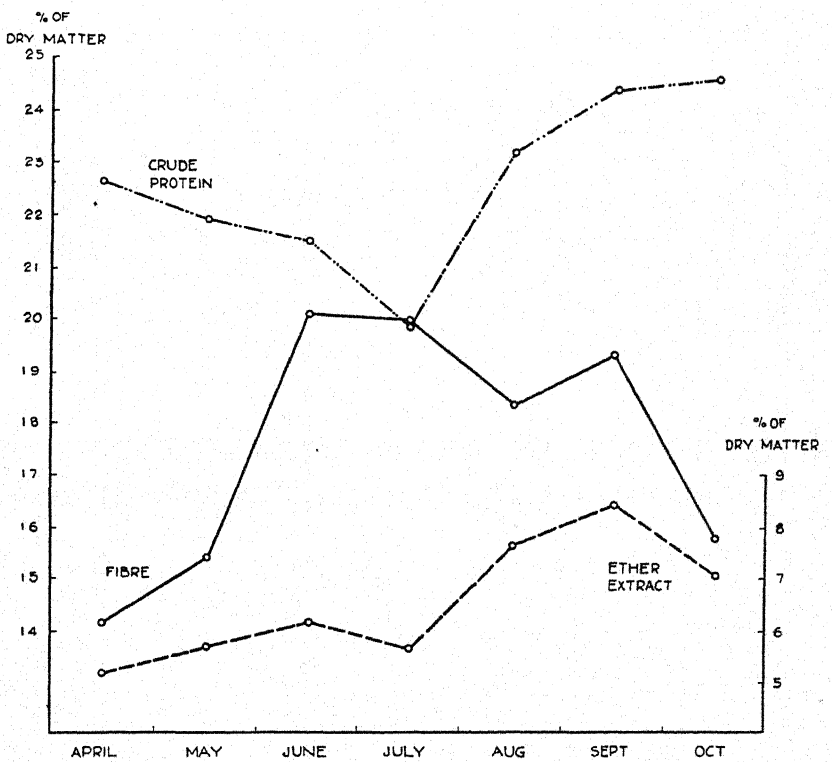


Fig. 1. Graphs showing seasonal variations in composition of dry matter of herbage on intensive paddocks at grazing stage. Tollesby Farm, 1926.

stems were present. From the end of July onwards a very plentiful supply of young leafy grass was obtained. The paddocks were grazed by sheep during March, and by the dairy herd from the beginning of April until the end of October.

On the non-intensive paddock, growth commenced noticeably later and finished earlier than on the intensive paddocks; the bulk of herbage at each grazing was also much less than in the latter cases.

(iii) 1927. *Melchet Court.*

Following a cold, wet spell in early spring, dry, sunny conditions prevailed from mid-April until mid-June; the remainder of June and the

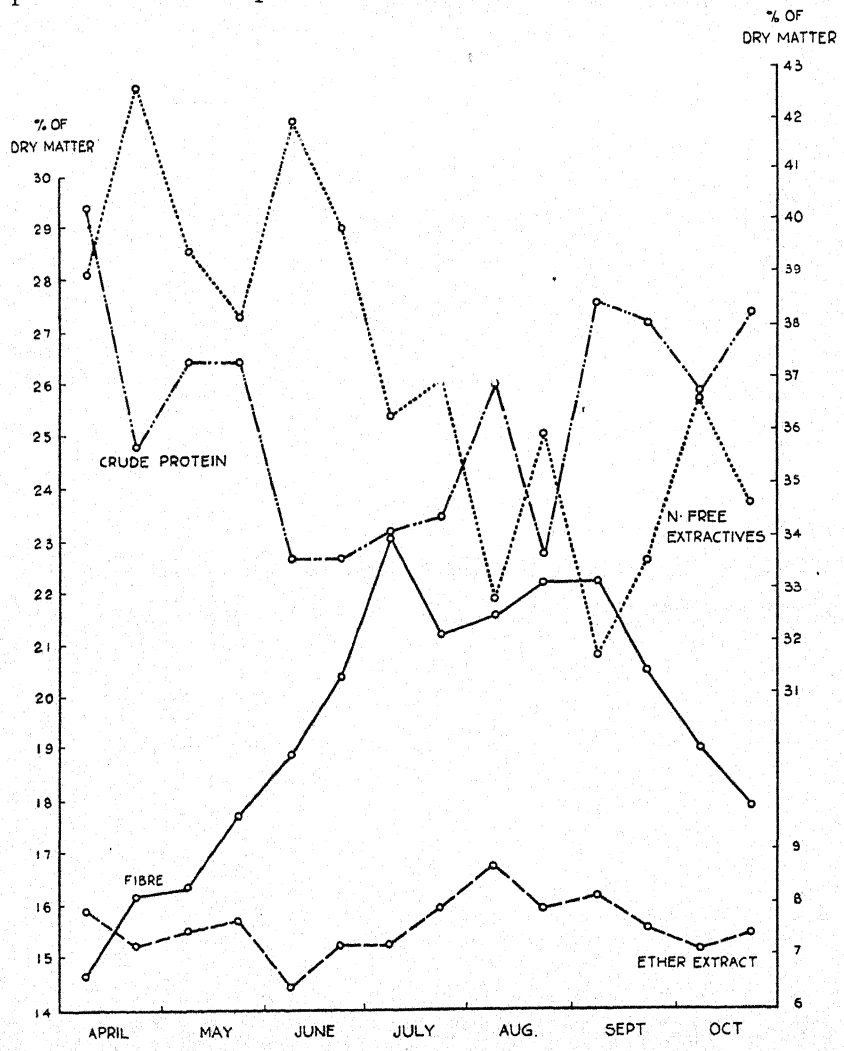


Fig. 2. Graphs showing seasonal variations in composition of dry matter of herbage on intensive paddocks at grazing stage. Tollesby Farm, 1927.

next three months, however, were abnormally wet. An adequate supply of young grass was obtained during April and the first half of May; from then until the end of June, though, the grass was of short growth and

many flowering stems sprang up, whilst at mid-June the paddocks were practically bare. From the beginning of July onwards, there was an abundant supply of young, leafy herbage.

The paddocks were grazed by the dairy herd from the beginning of April until the end of September, except for the period of shortage in June.

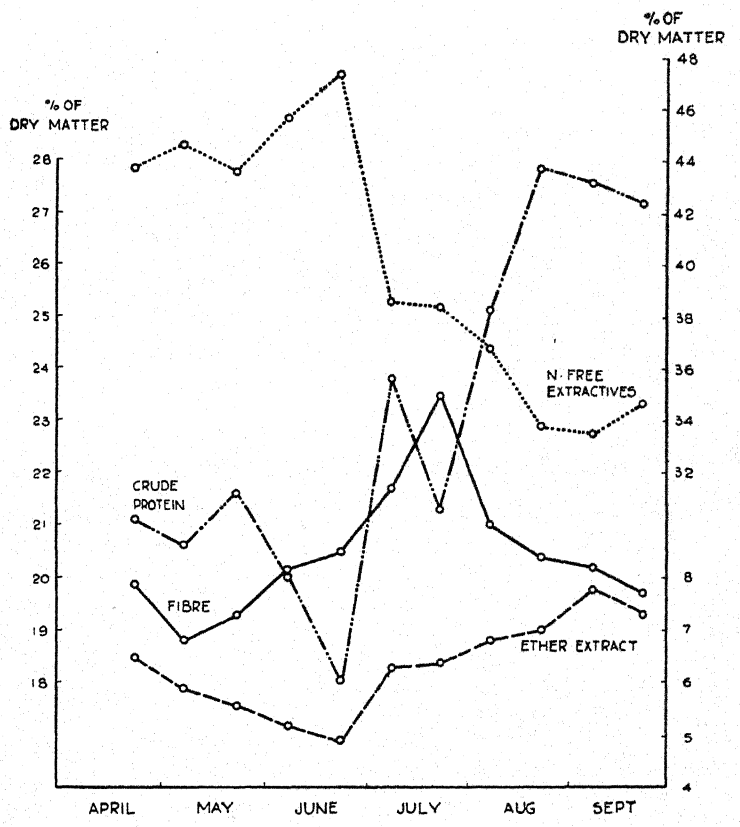


Fig. 3. Graphs showing seasonal variations in composition of dry matter of herbage on intensive paddocks at grazing stage. Melchet Court, 1927.

(iv) 1929. *Jealott's Hill.*

Following an exceptionally dry and cold winter, the spring and summer of 1929 were abnormally dry and sunny. The grazing season was, in consequence, a very short one, extending only from the beginning of May until the middle of September. Although the grass was in general short, the paddocks provided continuous grazing for the dairy herd

during this period and held out noticeably better against the drought than neighbouring pastures not under the Intensive System of Management.

The management of the paddocks was carried out at each centre to satisfaction and in all cases the pastures showed a marked improvement under the Intensive System treatment.

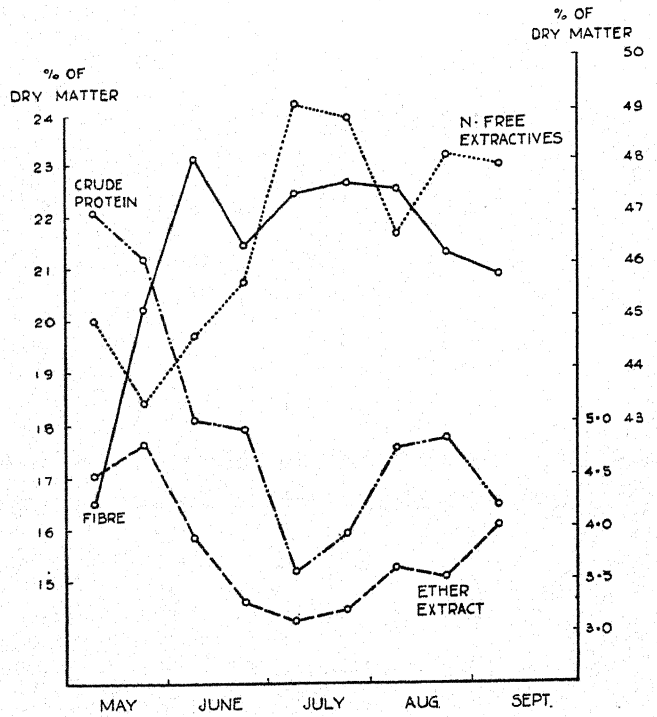


Fig. 4. Graphs showing seasonal variations in composition of dry matter of herbage on intensive paddocks at grazing stage. Jealott's Hill, 1929.

Chemical composition data.

The average composition of the dry matter of the herbage at the grazing stage, during successive periods of the grazing season, in respect of the organic constituents, is shown graphically for the respective centres and seasons in Figs. 1 to 4, whilst the average seasonal composition values are given in Table II.

Table II. *Average seasonal composition of dry matter of herbage at grazing stage.*

	Intensive paddocks				Non-intensive paddock
Year ...	1926	1927		1929	1927
Centre...	Tollesby Farm (%)	Tollesby Farm (%)	Melchet Court (%)	Jealott's Hill (%)	Tollesby Farm (%)
Ether extract	6·5	7·6	6·3	3·8	7·0
Fibre	17·5	19·3	20·5	21·2	19·0
Crude protein	22·6	25·3	23·1	18·0	22·6
Nitrogen-free extractives	—	37·1	40·1	46·6	41·3
True protein	19·6	22·5	20·8	14·5	19·9
Ratio true : crude protein	0·87	0·89	0·90	0·81	0·88
Dry matter in fresh herbage	18·6	18·0	—	27·2	21·1

DISCUSSION.

The main results of the work may be stated briefly as follows:

1. The dry matter of pasture as grazed under the Intensive System of Grassland Management is a concentrated feeding stuff, rich in protein and comparatively low in fibre; the actual composition, however, is subject to seasonal and yearly variation.

2. Yearly variations in composition were manifested mainly in the constituents protein, ether extract and nitrogen-free extractives; in the abnormally dry season of 1929, compared with the normal and very wet seasons of 1926 and 1927 respectively, the first two constituents were noticeably diminished in amount and the last constituent correspondingly increased. The average fibre content, however, was only slightly higher in the dry than in the wet season. The average content of crude protein in the dry matter ranged in 1926 and 1927, from 22·6 to 25·3 per cent.; in 1929 the value was 18·0 per cent.

3. Seasonal variations in composition consisted in a decrease in the protein and a corresponding increase in the fibre content during mid-season, with a recovery in both respects later. The constituents ether extract and nitrogen-free extractives also showed definite seasonal variations, the nature of which differed with centre and season.

4. The ratio true : crude protein showed no appreciable seasonal variation, but was noticeably lower in the dry season of 1929 than in the wetter seasons of 1926 and 1927.

5. The chemical composition of the herbage was a marked reflection of both its habit of growth, and its productivity, and these were in turn both mainly dependent on the rainfall. Adequate rainfall produced an

abundant supply of young leafy herbage of high nutritive value whilst under drier conditions less herbage was produced, its habit of growth was more stemmy and its nutritive value lower; in all cases, however, the natural tendency of grass to develop flowering stems at the normal time of flowering, with a consequent decrease in nutritive value, was manifested.

6. Comparing the intensive paddocks with a non-intensive paddock of good quality, grazed rotationally at Tollesby Farm in the wet season of 1927, the former yielded much more herbage than the latter and at the same time contained throughout the season rather higher percentages of protein and ether extract; the non-intensive pasture contained a correspondingly larger proportion of nitrogen-free extractives.

The results have established, for pastures farmed under the Intensive System, the recent findings of Woodman and his associates (1, 2, 3, 4) at the Cambridge University School of Agriculture, for unmanured pastures, in respect of the high nutritive value of young, leafy pasture; a comparison of the more important average seasonal composition data of the Cambridge and present investigations is set out in Table III. Woodman (1, 2) found that the average seasonal crude protein content of unfertilised pasture, cut at weekly intervals, was 24·7 per cent. of the dry matter of the herbage. Under a system of fortnightly cutting (3), the value was 23·5 per cent. and when the interval was increased to three weeks (4), 21·1 per cent. The present investigations have shown that, under a system of intensive manuring and rotational grazing, these values may be maintained and even exceeded when the pastures are grazed at a stage of growth which ranges usually between three and five weeks.

Table III. *Comparison of average seasonal chemical composition data, Cambridge and Intensive System investigations (on basis of dry matter).*

	Cambridge investigations (1, 2, 3, 4)				Intensive system investigations *				
Year...	1925	1926	1927	1928	1926	1927			1929 †
						Tollesby Farm			
	Light pasture cut weekly (%)	Heavy pasture cut weekly (%)	Light pasture cut fortnightly (%)	Light pasture cut 3-weekly (%)	Tollesby Farm Intensive paddocks (%)	Intensive paddocks (%)	Non-intensive paddock (%)	Melchet Court Intensive paddocks (%)	Jealott's Hill Intensive paddocks (%)
Crude protein	24·7	24·7	23·5	21·1	22·6	25·3	22·6	23·1	18·0
Ether extract	5·3	6·5	6·5	6·0	6·5	7·6	7·0	6·3	3·8
Fibre	15·4	18·3	15·9	17·2	17·5	19·3	19·0	20·5	21·2
Nitrogen-free extractives	44·8	41·2	44·5	46·7	—	37·1	41·3	40·1	46·6

* Pastures grazed usually at three to five weeks' stage of growth.
† Abnormally dry conditions prevailed throughout the season.

Further, intensive manuring not only increases the nutritive value of the pastures but it greatly increases their productivity. Thus in 1927 the dry matter of the herbage from the intensive paddocks at Tollesby Farm, not only contained an average of 25·3 per cent. of crude protein, compared with 22·6 per cent. for that from the non-intensive paddock, but the seasonal milk productions were an average of 713 gallons per acre in the former, compared with 409 gallons per acre in the latter case.

The high protein content of intensive pasture raises an important practical point, namely that of supplementary feeding. Woodman (1, 2, 3, 4) has shown that the digestibilities of the constituents of young grass are very high and that in view of its high content of digestible protein, such grass is, by itself, an unbalanced food for most classes of stock. Cattle on intensive pastures should therefore be controlled in the quantity of herbage they are allowed to consume, and a part of their feed be given in the form of a supplementary food rich in carbohydrate. Further, it has been noticed in the present investigations and has also been reported by Gardner and his associates (5), that the first-line stock are given to "tipping" the grass when turned on to a fresh paddock, thus consuming herbage particularly rich in protein.

The question of supplementary feeding is of greatest importance when the nutritive value of the herbage is highest, namely in the early and late months of the season. At these times of the season also, the proportion of indigestible fibre in the herbage is very low and it is advisable, therefore, in the early and late months of the season, to ensure that the stock obtain sufficient roughage in their diet, by including in their supplementary ration some fibrous food.

The actual stage at which pasture is grazed under the Intensive System, cannot be determined by any fixed rules, but must in practice be subject to variation according to climatic and management conditions. Generally speaking the herbage should be grazed at a height of about 4 inches, which will be attained usually during a growth interval of from three to five weeks, but under some circumstances the height may be more or less than 4 inches, or the growth interval be outside the three to five weeks' range. For instance, during the very wet summer of 1927, on several occasions the grass reached a height of six inches and over during three to four weeks; this herbage, however, had a crude protein content of 25 per cent. of its dry matter and was entirely suitable for grazing. On the other hand, in the dry season of 1929, the herbage frequently did not reach a height of even three inches during a period of four to five weeks. The stage of growth to which the data recorded in these investigations refer therefore, was not rigidly fixed at a definite height or time interval,

but was subject to certain variation according to climatic and management conditions. Such variation may account in some measure, of course, for the seasonal variations observed in the chemical composition of the herbage.

The values for the dry matter content of the fresh herbage present two features of interest, firstly, the difference between the intensive and non-intensive paddocks, and secondly, the comparatively low moisture content of the herbage in the dry season of 1929. Over corresponding periods, the average dry matter content of the intensive herbage at Tollesby Farm, in 1927, was 17·1 per cent. compared with 21·1 per cent. for the non-intensive herbage. These figures compare with an average value of 27·2 per cent. for the intensive paddocks at Jealott's Hill in the dry season of 1929. A higher proportion of moisture in intensive, compared with non-intensive pastures, has been reported also by Archibald and Nelson (6).

In conclusion, brief reference is made to the published results of other recent pasture investigations. Gardner and his associates (5), for pastures farmed under the Intensive System in 1927 and 1928, at the Hertfordshire Institute of Agriculture, "Oaklands," St Albans, report an average crude protein content of 17·7 per cent. for the dry matter of the herbage from intensive paddocks, compared with 15·5 per cent. for non-intensive paddocks grazed rotationally. These values are appreciably lower than those obtained in the present investigations, but it appears that the herbage at "Oaklands" was allowed to reach a more advanced stage of growth before being grazed: Gardner, in fact, states "the average age of the grass when sampled was 35 days." Also, it may be mentioned that Gardner's figures for intensive pasture are based on data from only two paddocks.

Griffith and Phillips (7) have published data for one intensive paddock of pasture farmed under the Intensive System in 1928, on the farm of the University College of Wales, Nantcellan, Aberystwyth. The average crude protein content of the dry matter of the herbage was 18·9 per cent. Very dry conditions, however, prevailed from February until the middle of June, and not until July was the rainfall sufficient for the successful operation of the Intensive System.

Archibald and Nelson (6) report a value of 17·5 per cent. for intensive paddocks compared with 13·8 per cent. for a non-intensive paddock rotationally grazed, in Intensive System investigations under American conditions in 1928, on the Massachusetts Agricultural College Farm.

Investigations have been carried out also on pastures and individual

species of grasses cut at frequent intervals to simulate rotational close grazing. In addition to the work of Woodman and his associates (1, 2, 3, 4) already referred to, mention may be made of that of Shutt, Hamilton and Selwyn (8, 9) at the Dominion Experimental Farm, Ottawa, Canada, of Enlow and Coleman (10) at the Florida Agricultural Experiment Station in America, and of Fagan and his colleagues (11, 12, 13, 14) at the Welsh Plant Breeding Station, Aberystwyth. The general findings of these workers are in agreement with those of Woodman and of the present investigations. The results show that the nutritive value of pasture is increased under systems of frequent cutting, and that both the productivity and nutritive value are increased by manuring. Fagan's work has illustrated also the marked superiority of the nutritive value of the leaf over that of the stem.

SUMMARY.

1. Herbage from pastures managed under the Intensive System of Grassland Management, and representing three to five weeks' growth, from three centres in three seasons, was closely comparable with that obtained by other workers from "pasture cuts" at one- to three-weekly intervals from unmanured pastures, in respect of the chemical composition of its dry matter, which is, in fact, a concentrated feeding stuff of narrow nutritive ratio and low in fibre content.

2. The nutritive ratio is somewhat wider in a dry than in a wet season, but the average fibre content is not markedly different.

3. During the grazing season, drought or the early summer flush period produce some reduction in protein content and an increase in fibre content, with a recovery following the cessation of the drought or after the flush period respectively.

4. The variations noted under (2) and (3) above are a reflection of the productivity and habit of growth of the herbage. An abundant growth of young leafy herbage has a higher nutritive value, weight for weight of dry matter, than the more stemmy growth which is liable to be produced in early summer or under conditions of drought.

5. The protein content of the dry matter of herbage from pasture receiving intensive manuring including nitrogenous fertilisers, was higher than that from pasture not so treated. Thus the higher milk-producing power of the former pasture may be ascribed in part to improved composition of the herbage as well as to a higher yield of herbage.

6. The bearing of the results on the question of supplementary feeding is discussed.

ACKNOWLEDGMENT.

For the facilities given for undertaking the investigations at Melchet Court and at Tollesby Farm, thanks are due to Lord Melchett and to Mr W. Brunton respectively. Thanks are due also to Mr J. Procter and to Mr N. Pollard, to the former for the collecting of the samples of herbage at Melchet Court and to the latter for the carrying out of much of the analytical work.

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(*Received July 25th*, 1930.)

NUTRITIVE VALUE OF PASTURE.

VI. THE UTILIZATION BY SHEEP OF MINERAL-DEFICIENT HERBAGE.

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Introduction.

A notable contribution to our knowledge of the factors which influence the nutritive value of grass has been made by Dr J. B. Orr in a recent publication dealing with minerals in pastures and their relation to animal nutrition (1). The treatise was primarily the outcome of the deliberations of a sub-committee appointed in 1926 by the Civil Research Committee of the Cabinet to consider and report on the relationship between the mineral content of pastures and their nutritive value. From the initial enquiries instituted by this sub-committee, it was evident that malnutrition in cattle and sheep arising from deficiency of minerals in grass was widespread in the pastoral areas of the Empire, and that the subject, which was of great economic significance, warranted close and systematic investigation. A report to this effect was duly forwarded to the Civil Research Committee, on whose further recommendation grants were made by the Empire Marketing Board in aid of a comprehensive scheme of investigations into the mineral aspects of pastures within the Empire. A two-fold scheme of work was adopted, actual investigations in selected grassland areas being supplemented by a detailed search of the literature dealing with every phase of the subject.

In summarizing the results of these investigations, Dr Orr begins with an explanation of the economic importance of grassland and then passes on to trace the development of scientific methods for investigating the problems of pastures. This is followed by a minute survey of the results of many investigations into the mineral composition of both good and poor pastures in the British Isles and in various parts of the world. The factors which influence the mineral content of pastures are also considered in the light of present knowledge. Dr Orr then proceeds to deal with various forms of disease which have been attributed to deficiency of minerals in pasture herbage. The deficiency diseases of grazing animals in Europe, Africa, Australasia, America and Asia are described, and the results of

investigations into the causes of such diseases are summarized and critically discussed. The author treats also of the prevention of deficiency diseases in pasturing stock, either by the direct administration to the animal of the deficient minerals, or by the enrichment of the pastures through the application of mineral fertilizers to the soil. It is shown that the prevention of disease by these measures leads also to an increased rate of growth in animals and to an increased production in adult females.

In an appreciative review of this volume, the late Prof. T. B. Wood (2) raised the question as to whether the failure of animals to thrive on these deficient pastoral areas may not sometimes be due as much to the energy-deficient character of the herbage as to its deficiency in minerals. The starch value of such herbage may be so low that animals, even when consuming it to the limit of appetite, may not be able to secure sufficient net energy to permit of normal development. The further question also arises as to the method whereby mineral deficiencies cause the symptoms of malnutrition. These may arise conceivably from four causes: (1) The digestibility of the organic constituents of the herbage may be depressed in the absence of an adequate supply of minerals; (2) the lack of sufficient mineral matter of proper quality may confer an unpalatable character on the herbage, a circumstance which might react adversely on the appetites of animals subsisting on such herbage; (3) the physiological utilization of the digested nutrients may not proceed normally, if the amount of minerals in the herbage is deficient; (4) the mineral deficiency may prevent the normal development of the skeletal structure. Of this, there can of course, be no doubt, the question at issue being solely the extent to which the first three factors may also operate in the production of physiological disturbances in animals subsisting on mineral-deficient herbage.

In the investigation to be outlined in the present communication, an attempt has been made to secure information which might furnish an answer to these and kindred questions. A detailed study has been made of the utilization by sheep of a sample of mineral-deficient herbage, which was kindly supplied for the purpose by Dr. J. B. Orr, of the Rowett Research Institute. The amount of material available, namely, about 25 cwt., did not permit of prolonged feeding trials on more than two animals; accordingly, two pure-bred Suffolk wethers, aged about 15 months at the commencement of the investigation, were employed. The herbage, as will be shown later, had reached the fibrous stage of maturity associated with poor meadow hay. This, from the standpoint of the

present investigation, was a distinct disadvantage, since grazing animals would presumably secure the herbage in a much younger stage of growth, when composition and digestibility would have been superior to that of the mature forage. It was obvious that more conclusive results would have been obtained if it had been possible to conduct the trials in the actual mineral-deficient pastoral area, so that, by applying the Cambridge technique of cutting at stated intervals, the value of the herbage in its different stages of growth could have been investigated.

The general scheme of the trials was as follows:

(1) Preliminary period of feeding, during which the appetites of the animals on the diet of mineral-deficient herbage were assessed.

(2) Period of controlled feeding in metabolism crates, for the purpose of determining the digestibility and nutritive value of the herbage, and for measuring the nitrogen and mineral balances in the animals (period 1).

(3) A prolonged period of generous feeding on the herbage, with a view to noting changes of appetite, live-weight and condition in the sheep. It was thought that the results obtained in period 1 of the metabolism trials might be misleading, since at this early stage the animals would possess abundant body reserves of minerals. Such reserves might conceivably be drawn on to make good the deficiency of minerals in the diet, and by such adjustment the influence of dietary mineral deficiency on the digestion and utilization of the herbage might be masked. A long period of feeding on the herbage might be necessary, therefore, to afford the animals an opportunity of manifesting disturbances arising from mineral deficiency. It must be borne in mind that under natural conditions, such animals would be required to subsist on the herbage over very long periods.

(4) A second period of controlled feeding in metabolism crates, during which time measurements of digestibility and of nitrogen and mineral balances were again carried out (period 2).

(5) A short period of feeding on mineral-deficient hay plus added minerals, followed by measurements of the influence of the mineral supplement on the digestion and utilization of the herbage (period 3).

(6) A prolonged period of feeding on the herbage plus minerals, in order to ascertain whether the added minerals brought about any improvement in the appetite, live-weight and condition of the sheep. This period of feeding was followed by further measurements of the digestibility of the herbage, when supplemented with minerals, and of the nitrogen and mineral balances (period 4). Here again it was assumed that an improvement in assimilation, consequent on an addition of

minerals to the deficient diet, might only be apparent after a long period of feeding.

(7) A final period of feeding, in which digestion and balance data were obtained when both the mineral and oil deficiencies (see Table I) in the herbage were corrected by the addition of a mineral mixture and of cod-liver oil respectively (period 5). It is recognised that when a ration is deficient or badly balanced in respect of minerals, the addition of cod-liver oil to the ration may lead to greater assimilation and retention of lime, and consequently to an improved rate of growth. The present trials afforded an opportunity of testing this finding.

Botanical composition of mineral-deficient herbage.

The writers are indebted to their colleague, Mr S. F. Armstrong, M.A., for the following information respecting the botanical character of the sample of mineral-deficient herbage:

Rather more than half the bulk of the herbage consisted of purple melick grass (*Molinia cærulea*), a plant which is common on damp moors and peaty soils. This species had reached the full flowering stage and some immature seeds had been formed. The proportion of flowering stem, however, was small, the bulk of the grass consisting of very tough, hard, dry leaves, 8 to 12 inches in length. Next in order of abundance came brown bent grass (*Agrostis canina*), which formed about 20 per cent. of the total herbage. It was in the late flowering and early seeding stages. Sheep's fescue (*F. ovina*), in the flowering stage, constituted about 5 per cent. of the sample, whilst *very small quantities* of the following species were also present: Yorkshire fog (flowering stage), sweet vernal grass (full of ripe seed), rough stalked meadow grass (flowering stage) and fiorin (flowering stage).

The herbage, having been cut close to the ground, apparently by scythe, included a fair proportion of dead leafage from the previous year's growth and a small amount of mosses. It is important to note that the material contained neither clovers nor any other kind of leguminosae.

Chemical composition of mineral-deficient herbage.

The composition of the herbage samples for the several digestion periods is shown in Table I. The data are given on the basis of dry matter. Moisture determinations on the herbage ranged from 11·50 to 13·25 per cent.

Table I. *Chemical composition of period samples of mineral-deficient herbage (on basis of dry matter).*

	Period 1 %	Period 2 %	Period 3 %	Period 4 %	Period 5 %	Mean of all periods %
Crude protein	12·93	13·00	12·76	13·37	12·95	13·00
Ether extract	2·20	1·90	2·00	1·72	1·69	1·90
N-free extractives	46·93	46·72	46·92	45·85	45·52	46·39
Crude fibre	34·37	34·98	34·93	35·51	35·90	35·14
Ash	3·57	3·40	3·39	3·55	3·94	3·57
Silica	0·78	0·56	0·56	0·33	0·68	0·58
Silica-free ash	2·79	2·84	2·83	3·22	3·26	2·99
Lime (CaO)	0·29	0·26	0·27	0·27	0·27	0·27
Phosphoric acid (P_2O_5)	0·24	0·28	0·21	0·27	0·25	0·25
Potash (K_2O)	1·13	1·18	1·22	1·18	1·23	1·19
Soda (Na_2O)	0·20	0·11	0·15	0·16	0·12	0·15
Chlorine (Cl_2)	0·44	0·45	0·41	0·43	0·44	0·43
Magnesia (MgO)	0·39	0·31	0·29	0·30	0·29	0·32
True protein	12·65	12·92	12·59	12·74	12·44	12·67
"Amides"	0·28	0·08	0·17	0·63	0·51	0·33

Comments on Table I.

The figures in Table I show clearly that the mineral-deficient herbage was cut at a stage of maturity suitable for hay-making, a conclusion which is borne out also by the botanical examination of the material. Comparisons as to composition and feeding value, therefore, should be made with normal herbage cut for hay, and not with normal pasturage. An average sample of meadow hay, on the dry matter basis, contains 11·3 per cent. of crude protein, 2·9 per cent. of ether extract, 47·9 per cent. of N-free extractives, 30·7 per cent. of crude fibre and 7·2 per cent. of ash (3). The experimental herbage, therefore, contained, in comparison with medium meadow hay, approximately the same amount of N-free extractives but rather more crude protein. In addition, it was richer in fibre but poorer in ether extract. The most striking feature from the standpoint of the present investigation, however, was its poorness in respect of ash, the herbage containing only half as much mineral matter as is present in medium meadow hay.

The data in Table I reveal the nature of the mineral deficiencies. Both lime and phosphoric acid, particularly the former, were present in sub-normal amount. It is not easy to decide what is a reasonable figure for the lime content of average meadow hay, since the amount is influenced by the proportion of clover in the sample. There are grounds, however, for accepting a value higher than one per cent. of the dry matter. Klimmer and Schmidt (4), in summarizing the analytical data obtained by a number of investigators, give a series of values ranging from 0·95 to

1·60 per cent. of lime in normal hay, with a mean value of 1·20 per cent. of the dry matter. The percentages of phosphoric acid in the same samples varied from 0·42 to 0·81 per cent., with a mean value of 0·53 per cent. In investigations at Cambridge (5), samples of hay from a good field in two different seasons contained 1·37 and 1·27 per cent. of lime, and 0·62 and 0·72 per cent. of phosphoric acid respectively (dry matter basis). Since similar values have been obtained by numerous other investigators, there can be no doubt that the experimental hay sample, with its 0·27 per cent. of lime and 0·25 per cent. of phosphoric acid, was seriously deficient in respect of both these constituents.

Judged on the basis of available data, the herbage appeared also to be deficient in soda, the value 0·15 per cent. for this constituent contrasting unfavourably with the following results for the amount of soda in the dry matter of good hay: 0·39 per cent. (6) and 0·34 per cent. (7). In this connection, however, it should be recalled that grass generally tends to be poor in soda, and that the custom of giving grazing animals access to rock salt for the purpose of supplying chlorine is also sound from the standpoint of supplementing the low percentage of soda in the herbage.

Although the percentage of potash in the herbage was somewhat lower than is usually found in good meadow hay, the value 1·19 per cent. was high enough to obviate any danger of potash deficiency. Neither was there any deficiency in respect of magnesia, the figure 0·32 per cent. for the experimental sample comparing satisfactorily with the values 0·38 per cent. (8) and 0·23 per cent. (7) for good meadow hay and 0·32 per cent. (9) for timothy hay. Figures varying from 0·32 to 0·53 per cent. of the dry matter, with a mean value for three separate samples of 0·42 per cent., are given by Fagan (10) for the chlorine content of meadow hay. The value 0·43 per cent. of chlorine for the experimental herbage agrees well with Fagan's average value and suggests that there was no serious deficiency in respect of this constituent.

It may be concluded, therefore, that lime shortage represented the main mineral deficiency in the herbage, and that, in addition, there was a serious deficiency of phosphoric acid. The supply of soda in the herbage was also on the low side.

Period of feeding on mineral-deficient herbage without mineral supplement.

For some weeks prior to the commencement of the trials, each sheep had been receiving a daily ration of 1300 gm. of sainfoin hay. During this time, they had been given opportunities of becoming thoroughly ac-

customed to wearing the light digestion harness and occupying the metabolism crates, a precaution which has been shown to be necessary before venturing on experiments connected with metabolism. Between July 30 and August 4, 1929, the animals were brought gradually on to the diet of mineral-deficient herbage, the latter being fed in the chaffed condition throughout the duration of the trials. From August 5 to September 10, the daily ration consisted of 1200 gm. of the chaffed hay. Accounts were kept of the amount of hay consumed daily, and the animals were weighed at short intervals.

On August 19, the sheep were placed in metabolism crates and the first digestion period was begun on August 26. After the completion of this period of measurements, the sheep were removed from the crates, weighed and allowed the freedom of commodious pens, where, from September 10 to October 28, they were given a daily ration of 1500 gm. of the mineral-deficient herbage, observations of food consumption and live-weight changes being made throughout the period. On October 29, the sheep were again placed in the metabolism crates, the ration being reduced to 1200 gm. of the herbage, and a second digestion period of 14 days' duration was begun on November 5.

It will be noted, therefore, that the sheep subsisted on the mineral-deficient herbage, unsupplemented by minerals, for a total period of 15 weeks. A summary of the data obtained in respect of appetite and live-weight is given in Table II, whilst in Tables III and IV are recorded the results which were obtained in periods 1 and 2 of the digestion trials.

Table II. *Records of appetite and live-weight during the period of feeding on mineral-deficient herbage alone.*

		Sheep IX			Sheep X		
		Hay consumed. Av. per day	Live-weight		Hay consumed. Av. per day	Live-weight	
Date (1929)	Hay supply gm.	gm.	st.	lb.	gm.	st.	lb.
Aug. 5 to Aug. 11	1200	1168	7	13	978	8	4
Aug. 12 „ Aug. 18	1200	1191	7	13	1044	7	13½
Aug. 19 „ Aug. 25*	1200	1003	—	—	1054	—	—
Aug. 26 „ Sept. 9*	1200	766	6	9½	1125	8	2½
Sept. 10 „ Sept. 16	1500	1132	7	8	1282	8	0
Sept. 17 „ Sept. 23	1500	1291	7	10	1295	8	8
Sept. 24 „ Sept. 30	1500	1363	7	11	1329	8	8
Oct. 1 „ Oct. 7	1500	1363	7	11½	1322	8	7½
Oct. 8 „ Oct. 14	1500	1325	7	11½	1345	8	10½
Oct. 15 „ Oct. 21	1500	1365	7	12½	1325	8	7½
Oct. 22 „ Oct. 28	1500	1359	7	13	1366	8	13
Oct. 29 „ Nov. 4*	1200	1185	—	—	1177	—	—
Nov. 5 „ Nov. 18*	1200	1186	7	2½	1185	8	9

* Sheep in metabolism crates.

Table III. *Summary of digestion coefficients of mineral-deficient herbage (periods 1 and 2).*

	Period 1 (Aug. 26 to Sept. 9)			Period 2 (Nov. 5 to Nov. 18)			Poor meadow hay (3)
	Sheep IX	Sheep X	Mean	Sheep IX	Sheep X	Mean	
	%	%	%	%	%	%	%
Organic matter	46·34	44·61	45·5	46·65	49·43	48·0	48·1
Crude protein	49·47	49·53	49·5	49·93	54·11	52·0	45·3
Ether extract	15·09	4·22	9·7	8·65	2·92	5·8	33·3
N-free extractives	39·59	38·01	38·8	41·42	42·29	41·8	50·5
Crude fibre	56·45	54·33	55·4	54·49	59·73	57·1	46·6

Table IV. *Digestible nutrients and starch equivalent in mineral-deficient herbage (dry matter basis).*

	Period 1	Period 2	Mean of periods 1 and 2	Poor meadow hay (3)
	%	%	%	%
Dig. crude protein	6·40	6·76	6·58	4·0
Dig. ether extract	0·21	0·11	0·16	0·6
Dig. N-free extractives	18·21	19·53	18·87	22·5
Dig. crude fibre	19·04	19·97	19·50	18·2
Total dig. organic matter	43·86	46·37	45·11	45·3
Starch equivalent*	23·74	25·77	24·75	22·9
Nutritive ratio	5·9	5·9	5·9	10·5
* If fed after chaffing	33·70	35·92	34·81	34·3

Comments on Tables II, III and IV.

Palatability of mineral-deficient herbage. A consideration of the data in Table II leads to the conclusion that the mineral-deficient hay was no more unpalatable than normal hay of similar fibre content. In preliminary tests with sainfoin hay, containing 33 per cent. of fibre on the basis of dry matter, it was discovered that the daily ration must be restricted to about 1300 gm. in order to ensure clean consumption. No greater difficulty was experienced in changing over from the diet of sainfoin hay to that of mineral-deficient hay than is usually encountered when going from one kind of ration to another. The change-over was effected gradually, increasing amounts of sainfoin hay being replaced by mineral-deficient hay on successive days. During this time, sheep X left small amounts of the mineral-deficient hay, while sheep IX left no residues of any kind, despite the fact that the mineral-deficient herbage had a much tougher texture than the sainfoin hay.

After a week's transition period, both sheep were receiving a daily allowance of 1200 gm. of the mineral-deficient herbage. During the first fortnight on this herbage, sheep IX consumed nearly the whole of the hay supplied, the average daily food residues amounting to no more than

20 gm. Sheep X, although consuming 1160 gm. of the hay on the first day, fell behind sheep IX in respect of appetite, leaving, on an average, a daily residue of 222 gm. during the first week, and 156 gm. during the second week.

At this stage (August 19), the sheep were placed in metabolism crates for the purpose of digestion measurements. The appetite of sheep X showed continuous improvement, the animal consuming, on an average, a daily ration of 1054 gm. of hay during the preliminary period of a week and 1125 gm. during the experimental fortnight. Sheep IX, on the other hand, began to decline in appetite after being in the crate for 5 days, so that its average daily consumption of food during the first week fell to 1003 gm. and further to 766 gm. during the next fortnight. It appeared to be in good health, however, and it is noteworthy that the faeces of both sheep were firm and normal throughout the whole period of feeding on unsupplemented hay.

From September 10 to October 28, the sheep were kept in neighbouring pens and supplied daily with 1500 gm. of the mineral-deficient hay (chaffed). The appetites of both sheep underwent distinct improvement during the first week after their removal from the metabolism crates, sheep IX averaging 1132 gm. of hay per day and sheep X 1282 gm. The data secured in the period between September 17 and October 28 may be taken as affording a reliable indication of the animals' capacity for consuming the mineral-deficient hay, since over this period the daily food consumption by both animals was remarkably similar and uniform, sheep IX averaging 1344 gm. and sheep X 1330 gm. of hay per day. This represented a mean daily food consumption, for both sheep, of 1162 gm. of dry matter.

The mean live-weight of the sheep, over the period under consideration, was 114 lb. It is customary to assume that a sheep of this live-weight is able to consume about 1700 gm. of dry matter per day (3). This figure, however, must be regarded as applying to outdoor feeding on rations containing an abundant supply of succulent food or green fodder. By comparison with other indoor sheep feeding trials, the daily consumption of 1162 gm. of dry matter in the form of coarse, bulky hay may be regarded as satisfactory, comparing favourably with the results of the preliminary period on sainfoin hay, when it was found that the sheep were able to consume, without leaving residues, a ration restricted to 1300 gm. (= 1131 gm. dry matter). In the Cambridge digestion trials of freshly-mown pasture grass, a food of undoubtedly high palatability, it has been found desirable, in order to ensure clean consumption, to limit

the daily ration to 4000 gm., an allowance containing anything from about 800 to about 1300 gm., according to the moisture content of the herbage.

It will be noted, from the data in Table II, that both sheep continued to eat the hay satisfactorily after they were again placed in the metabolism crates on October 29 for the second digestion trial. At this point, the daily ration was reduced to 1200 gm., and almost complete consumption of this amount was secured, the small amounts of residues consisting substantially of seed and fine, dusty material. It is fair to conclude, therefore, that the hay, although coarse and fibrous, was not unpalatable, at any rate not more so than any other kind of hay made from herbage of similar maturity and fibre content. The further conclusion follows that the deficiency of lime and phosphoric acid in the herbage leads to no depression of appetite in sheep subsisting on it, even after a long period of feeding. In its younger stages of growth, in which condition grazing animals would presumably secure it, such mineral-deficient herbage would have a higher palatability than that which characterized the mature, fibrous hay of the present investigation, and it is safe to rule out altogether the hypothesis that the failure of grazing animals to thrive on such pasturage is due to under-feeding on herbage which is unpalatable because of its mineral deficiencies.

It may be objected that the present trials were not a fair test of the palatability of the mineral-deficient herbage, since manifestly the sheep had perforce to consume the ration or go hungry. Against this, however, it may be pointed out that a similar condition obtains when animals are put out to subsist on mineral-deficient grassland. Attention may also be directed to the fact that no difficulty was encountered in bringing the sheep on to the diet of mineral-deficient hay after they had become accustomed to a diet of sainfoin hay of good palatability. Experience in investigations with sheep shows that it is a problem of great difficulty to induce sheep to eat a ration which is definitely unpalatable, even when the method of starvation is resorted to.

Digestibility of mineral-deficient herbage. The data in Table III show that the mineral-deficient hay was as digestible as a poor grade of meadow hay, that is to say, meadow hay which has been made from herbage in as fibrous a stage of growth as that of the mineral-deficient herbage. The mean digestion coefficient of the organic matter of the hay, for both sheep in period 2, was 48 per cent., the corresponding figure for poor meadow hay (fibre content = 39 per cent. of the dry matter) being 48·1 per cent. The comparison, therefore, does not support the contention that such

herbage is characterized by low digestibility on account of deficiency of mineral matter. As with normal, cultivated herbage, the digestibility of mineral-deficient herbage is a function of maturity, being influenced by the degree of lignification of the fibre, and not by the amount and character of its inorganic constituents.

It will be noted that in period 1, sheep IX digested the hay somewhat more efficiently than did sheep X, whereas the reverse was true in period 2. It is significant that the animals, after subsisting on the mineral-deficient hay for a period of 13 weeks, showed no falling off in their capacity to digest the hay. Indeed, in the case of sheep X, the digestion coefficient of the organic matter was distinctly higher in period 2 (49·4 per cent.) than in period 1 (44·6 per cent.). If mineral-deficiency could be responsible for causing a depression of the digestibility of the organic constituents of a ration, then this might have been expected to be manifested during the second digestion trial, since during this period, not only was the ration deficient in mineral matter, but the sheep themselves, over the previous period of 13 weeks, had been in a condition of negative lime and phosphate balance.

It must be concluded that a deficit of lime and phosphoric acid in the diet does not depress digestibility. This may be explained on the following grounds: lime and phosphoric acid are not essential agents in the display of bacterial and enzymatic activity concerned with ruminant digestion; or, if they are, only very small amounts are required. (It should be noted here, of course, that lime salts play an essential part in the action of rennin on caseinogen, but this type of digestive activity does not arise in connection with the present problem.) Alternatively, if an abundant supply of lime and phosphoric acid is needed for normal digestion of the organic constituents of the diet, then it must be inferred that the organism is capable of supplying, from its own reserves, the necessary amounts of these minerals which are lacking in the food. If this be so, it would seem that the power is retained even after a long period of subsisting on a mineral-deficient diet, throughout which period the animal has been losing lime and phosphoric acid continuously from its body.

Further features of the data in Table III are worthy of special comment. It will be noted that the crude protein of the mineral-deficient hay was digested rather better than that contained in poor meadow hay. In this connection, it should be mentioned that the experimental herbage was much richer in this constituent than poor meadow hay, the respective percentages of crude protein, on the basis of

dry matter, being 13·0 and 8·8 per cent. The digestion coefficients for the ether extract of the mineral-deficient herbage varied considerably in the four separate digestibility determinations, but had one feature in common, namely, that all the values were very low, varying from 2·92 to 15·09 per cent., with a mean value of 7·7 per cent., comparing unfavourably with the corresponding figure of 33·3 per cent. for poor meadow hay. The lowness of the values and the lack of uniformity are not surprising in view of the low percentage of ether extract in the hay (viz. 1·9 per cent. of the dry matter) and the fact that the faeces always contain ether-soluble material of a metabolic character. The latter, in experiments on oil-deficient rations, may frequently amount to more than the ether-soluble portion of the ration and thereby give rise to an apparently anomalous result, namely, a negative digestion coefficient for the ether extract of the food (see results of period 4).

The most curious result in Table III is the finding that the fibre of the mineral-deficient herbage was distinctly more digestible than either the protein or the N-free extractives. A result of this character is not frequently encountered in digestion trials, although in the present investigation, it was obtained with both sheep in both digestion trials and, indeed, was again consistently given by both sheep in the three digestion trials carried out in the second and third stages of the work. Although this finding emphasizes the important rôle played by bacteria in the digestion of roughages by ruminants, it is difficult to understand why the N-free extractives in such herbage should run off more quickly than the fibrous constituent into an indigestible and unavailable condition.

Nutritive value of mineral-deficient herbage. The results in Table IV, summarizing the percentages of digestible nutrients and starch equivalent in the mineral-deficient hay, again bring out the similarity between the experimental herbage and meadow hay made from cultivated grass which has been mown at a late stage. The dry matter of poor meadow hay contains 45·3 per cent. of digestible organic matter (3), a figure in good agreement with the mean value of periods 1 and 2 for the mineral-deficient herbage, namely, 45·1 per cent. The main difference lies in the higher percentage of digestible protein, and consequently narrower nutritive ratio, which characterized the mineral-deficient hay. The latter was also deficient in respect of digestible ether extract.

The starch equivalent of the experimental herbage, namely, 24·75 lb. per 100 lb. of dry matter, is slightly higher than that of poor meadow hay, namely, 22·9 lb., a result in keeping with the fact that the mineral-deficient hay contained rather less fibre (35 per cent. of the dry matter)

than poor meadow hay (39 per cent.). The nutritive value of the mineral-deficient herbage, therefore, is such as would be anticipated for hay, of the same fibre content, made from herbage containing normal amounts of lime and phosphoric acid, a finding which points to the conclusion that the feeding value of herbage, from the standpoint of starch equivalent or net energy, is independent of mineral content. In other words, given two samples of herbage, the one deficient in lime and phosphoric acid and the other containing normal amounts, but both similar in respect of amount and kind of organic constituents, then a ruminant animal will be able to derive as much net energy from the mineral-deficient herbage as from an equal weight of the normal herbage.

That the mineral-deficient herbage was able to supply at least sufficient starch equivalent for maintenance is apparent from the weight data of the sheep between September 23 and October 28. Although uncertainty may attach to the result of an individual weighing, the records as a whole in the live-weight columns of Table II usefully indicate the progress of the sheep while receiving the mineral-deficient hay. Over the period of 5 weeks between September 23 and October 28, sheep IX increased in live-weight from 108 to 111 lb. and sheep X from 120 to 125 lb. If the herbage, after having been permitted to grow to a coarse and fibrous condition, is able to maintain live-weight in adult sheep, the inference may be drawn, on the basis of the Cambridge investigations into the relation between the feeding value of grass and its maturity, that in the earlier stages of growth, it would furnish not only the starch equivalent necessary for maintenance, but also that needed for growth. Failure of young animals to thrive on such herbage cannot, therefore, be attributed to a deficient supply of net energy or starch equivalent in the grass, but must definitely be ascribed to deficiency of inorganic constituents.

It is important to emphasize that the deficiency of minerals in the herbage did not prevent the sheep from maintaining live-weight, and even gaining weight slowly, over a period of several weeks. It must be borne in mind, however, that the animals were in adult condition, not requiring minerals for constructive purposes and containing in their bodies a good reserve supply of lime and phosphoric acid. In the case of growing animals, the mineral supply is of greater importance. Failure of young animals to thrive may be due to three kinds of dietary deficiency, namely, deficiency of starch equivalent, minerals and of vitamins. It has been demonstrated, however, that the mineral-deficient herbage, in the stage of growth at which it is consumed by grazing animals, contains sufficient

starch equivalent for both maintenance and growth. Further, it may be presumed that such herbage is as well supplied with vitamins as any other type of young herbage. The conclusion must be made, therefore, that malnutrition in young stock on pasturage of this character is due, simply and solely, to mineral deficiency.

Before concluding this section, it will be of interest to calculate the amount of starch equivalent which the sheep were consuming during the period of slowly increasing live-weight (September 23 to October 28). Sheep IX and sheep X consumed, on an average per day, 1355 and 1337 gm. of the herbage respectively, the mean daily consumption of dry matter for both sheep being 1171 gm. In assessing the starch value of this ration, the starch equivalent of the herbage must be taken as 34·81 per 100 lb. of dry matter, since the hay was chaffed before feeding. The mean daily starch equivalent consumption of the sheep, therefore, amounted to 0·9 lb., and on this they were able fully to maintain live-weight, the mean live-weight of the sheep over this period being 115 lb.

The most recent figure put forward for the daily starch equivalent requirement for maintenance in sheep is 1·26 lb. for the 100 lb. animal (11, 12), *i.e.* 1·39 lb. for a sheep of 115 lb. live-weight. It will be seen, therefore, that the sheep in the present experiment were able to maintain live-weight on a much smaller allowance of starch equivalent than that demanded by the standard figure for maintenance. Even if the starch equivalent of the herbage is increased by one-fifth, a procedure which the late Prof. T. B. Wood (3) maintained was necessary in the case of hays and straws, then the mean daily consumption of starch equivalent is increased to 1·08 lb., a figure, however, which still falls appreciably short of the standard figure. It must be concluded that either the standard mean value for maintenance in sheep is too high, or that the maintenance requirements of individual sheep, as is the case with bullocks, may vary considerably on either side of the standard value. It is significant that the results of the older German investigations established a lower value for the maintenance requirement in sheep, namely, 0·74 lb. of starch equivalent per day per 100 lb. live-weight (11). There is an obvious need for further enquiry into this question.

Period of feeding on mineral-deficient herbage plus mineral supplement.

The results brought forward in the preceding section led to the belief that the failure of stock to thrive in mineral-deficient pastoral areas could not be explained by assuming that the low mineral content was

responsible for a lack of palatability in the herbage and a consequent depression of appetite in the grazing animals. Neither was there any evidence that the shortage of minerals caused the herbage to be digested any less efficiently than normal cultivated herbage of similar maturity and organic composition. The amount of net energy which ruminant animals could derive from any form of herbage seemed to be independent of the mineral content and to depend solely on the amount and character of the organic constituents. It appeared justifiable to conclude that malnutrition on pasturage of sub-normal mineral content was due directly to the failure of the diet to supply the necessary inorganic materials for constructional purposes and for maintaining the normal balance of minerals in the blood and tissues.

In order to complete the chain of proof, it was decided to carry out a further period of feeding in which the deficient minerals in the herbage, namely, lime, phosphoric acid and soda, were brought up to the normal amounts for good meadow hay by the addition to the ration of a suitable mineral supplement. During this further period of feeding, observations were made of the influence of the added minerals on the appetites of the sheep and on the extent to which the sheep were able to digest the hay.

On November 18 the sheep were taken out of the metabolism crates after period 2 and given a daily ration of 1200 gm. of the mineral-deficient hay plus 30 gm. of the mineral mixture. The latter contained, per 100 gm., 34·8 gm. of tricalcic phosphate, 50 gm. of precipitated chalk and 15·2 gm. of common salt. Pure chemicals were employed, and the amounts of moisture, lime, phosphoric acid, soda and chlorine in the mineral supplement were determined in order to ensure the accuracy of the data for the mineral balances in the sheep. The mineral composition of the mixed ration in period 3, on the basis of dry matter, was as follows: total ash, 6·04 per cent.; lime, 1·55 per cent.; phosphoric acid, 0·64 per cent.; potash, 1·19 per cent.; soda, 0·37 per cent.; magnesia, 0·28 per cent.; chlorine, 0·65 per cent. That for periods 4 and 5 was very similar. The actual intake of minerals (*i.e.* supply minus residues) during these periods may be ascertained from the tables in the appendix, in which are recorded the data for the mineral and nitrogen balances.

Daily observations of the amount of food consumed by the sheep were made, and on November 25 the animals were again placed in the metabolism crates for the purpose of measuring the digestibility of the ration and securing data in connection with nitrogenous and mineral metabolism (period 3). It was noted that the faeces of the sheep re-

mained quite firm and normal during the period of feeding the mineral supplement.

After the completion of period 3 (December 12), the sheep were replaced in the pens and the ration was raised to 1500 gm. of the mineral-deficient hay plus 37·5 gm. of the mineral mixture. Daily observations of appetite and condition in the sheep were made over a period of 4 weeks, and on January 7, 1930, the animals were once more placed in the metabolism crates, the ration being reduced to 1200 gm. of hay plus 30 gm. of the mineral supplement. Further measurements of digestibility and mineral balances were carried out from January 14 to January 27 (period 4). At the end of this period, the ration was further enriched by the addition of cod-liver oil, the object being to bring up the percentage of ether extract in the ration to about 4 per cent. It was thought that the addition of the oil might have a favourable influence on the palatability and digestibility of the ration and on the assimilation of lime. The actual consumption of hay, minerals and oil during period 5 (February 7 to February 20) is shown in the appendix (period 5), the percentage of ether extract in the dry matter of the rations of this period being about 4·1 per cent.

Table V. *Records of appetite and live-weight during periods of feeding on* (1) *mineral-deficient hay plus minerals, and* (2) *mineral-deficient hay plus minerals plus cod-liver oil.*

	Food supply			Sheep IX			Sheep X		
				Food consumed. Av. per day	Live-weight		Food consumed. Av. per day	Live-weight	
Date 1929	Hay gm.	Minerals gm.	Cod-liver oil gm.	gm.	st.	lb.	gm.	st.	lb.
Nov. 5 to Nov. 18	1200	—	—	1186	7	2½	1185	8	9
Nov. 19 „ Nov. 25	1200	30	—	1097	7	4	1169	8	13½
Nov. 26 „ Dec. 12*	1200	30	—	1126	6	10	1209	9	2½
Dec. 13 „ Dec. 16	1500	37·5	—	1194		—	1178		—
Dec. 17 „ Dec. 23	1500	37·5	—	1255	7	6	969†	8	10
Dec. 24 „ Dec. 30	1500	37·5	—	1215	7	6	Refused to eat the ration ‡	8	5
Dec. 31 „ Jan. 6 1930	1500	37·5	—	1220	7	5		8	7
Jan. 7 to Jan. 11*	1200	30	—	1148		—	625		—
Jan. 12 „ Jan. 27*	1200	30	—	1109	6	9½	739	8	½
Jan. 28 „ Feb. 3	1200	30	28	896	6	10½	681	7	7½
Feb. 4 „ Feb. 20*	1200	30	28	1022	6	8	774	7	10
Feb. 21 „ Mar. 3	1200	30	28	1005	6	10	801	7	10

* Sheep in metabolism crates during following periods: Nov. 25 to Dec. 12 (period 3); Jan. 7 to Jan. 27 (period 4); Feb. 3 to Feb. 20 (period 5).

† Sheep X went off its food on Dec. 22 and ate only 307 gm. on this day. Its mean daily food consumption for this week up to Dec. 21 was 1169 gm.

‡ See comments on this table.

Table V is a record of the appetite and live-weights of the sheep during the periods of feeding on hay plus minerals and on hay plus minerals plus cod-liver oil. In Table VI are tabulated the digestion coefficients of the hay (mean for 2 sheep) obtained in all the five digestion periods. For the detailed results of the individual sheep, the appendix should be consulted. The percentages of digestible nutrients and starch equivalent in the mineral-deficient hay, as calculated from the results of the five digestion trials, are shown in Table VII.

Table VI. *Summary of digestion coefficients of mineral-deficient herbage in periods* 1, 2, 3, 4 *and* 5 (*Mean for* 2 *sheep*).

	Hay alone		Hay + mineral supplement		Hay + minerals + cod-liver oil
	Period 1 (Aug. 26 to Sept. 9) %	Period 2 (Nov. 5 to Nov. 18) %	Period 3 (Nov. 29 to Dec. 12) %	Period 4 (Jan. 14 to Jan. 27) %	Period 5 (Feb. 7 to Feb. 20) %
Organic matter	45·5	48·0	48·5	47·2	44·4*
Crude protein	49·5	52·0	54·3	55·4	51·3
Ether extract	9·7	5·8	23·4	—	— *
N-free extractives	38·8	41·8	40·8	37·4	34·3
Crude fibre	55·4	57·1	58·2	59·1	57·1

* Calculated on assumption that the cod-liver oil was fully digested and absorbed. For the whole ration, including the cod-liver oil, the digestion coefficients of the organic matter and the ether extract were 46·0 and 61·4 per cent. respectively (see Appendix, period 5).

Table VII. *Digestible nutrients and starch equivalents in dry matter of mineral-deficient herbage* (*periods* 1, 2, 3, 4 *and* 5).

	Hay alone		Hay + minerals		Hay + minerals + cod-liver oil
	Period 1 %	Period 2 %	Period 3 %	Period 4 %	Period 5 %
Dig. crude protein	6·40	6·76	6·93	7·41	6·64
Dig. ether extract	0·21	0·11	0·47	—	—
Dig. N-free extractives	18·21	19·53	19·14	17·15	15·61
Dig. crude fibre	19·04	19·97	20·33	20·99	20·50
Total dig. organic matter	43·86	46·37	46·87	45·55	42·75
Starch equivalent*	23·74	25·77	26·62	24·51	21·53
Nutritive ratio	5·9	5·9	5·9	5·1	5·4
* If fed after chaffing	33·70	35·92	36·75	34·81	31·94

Comments on Tables V, VI and VII.

Palatability of mineral-deficient herbage when supplemented with minerals. It is sometimes asserted that when animals have subsisted over a long period on mineral-deficient food and are then given access to a mixture containing the inorganic components which have been missing

from the diet, there is danger of their consuming, at the outset, excessive amounts of the minerals. Behaviour of this kind, however, was not shown by the sheep in the present trial, although, over a period of 15 weeks, they had been subsisting solely on the mineral-deficient herbage.

The introduction of the 30 gm. of mineral mixture into the ration of 1200 gm. of mineral-deficient hay did not cause the sheep to display any extra eagerness for their food. Indeed, on the first day, sheep IX showed a sudden decline in appetite, leaving 540 gm. of the ration. During the week which followed, however, its appetite improved again, so that its average daily food consumption amounted to 1097 gm. This, however, was smaller than the mean figure, namely, 1186 gm., for the previous week, when it was still receiving 1200 gm. of hay unsupplemented by minerals. Whilst in the metabolism crate during period 3 of the digestion measurements, sheep IX increased its mean daily food consumption still further to 1126 gm. There was no evidence, however, that the addition of the mineral supplement had caused the sheep to consume its ration with greater relish.

During period 3, sheep X left only a small amount of residues and consumed, on an average per day, 1209 gm. of the mineral-supplemented herbage. Again, however, it was not noted that the animal displayed any greater zest for its food than was shown when it was receiving mineral-deficient herbage only. Indeed, it seemed rather as if both sheep were almost indifferent to the presence of the mineral supplement, since they left residues which were much richer in minerals than the ration itself. For example, the ration during period 3 contained, on the dry matter basis, 6·04 per cent. of ash, including 1·55 per cent. of lime, whilst the food residues of sheep IX contained 20·68 per cent. of ash and 8·23 per cent. of lime, and those of sheep X, 51·65 and 21·45 per cent. respectively of these constituents. In other words, it was found that sheep IX rejected 29 per cent. of the total ash and 45 per cent. of the lime in the ration, whilst sheep X left 20 and 32·7 per cent. respectively (see Appendix). These figures do not afford any indications of an intuitive desire, on the part of the sheep, to secure as big a lime intake as possible, in order to make good the losses which they had suffered in the 15 weeks' period of feeding on the lime-deficient herbage.

In the 3½ weeks following the completion of period 3, when the ration of both sheep was increased to 1500 gm. of mineral-deficient hay plus 37·5 gm. of minerals, the mean daily food consumption of sheep IX rose to 1226 gm. This figure compares unfavourably with the corresponding figure for this sheep, namely, 1344 gm., when subsisting, under similar

conditions, on mineral-deficient hay alone. This period marked a serious falling off in the appetite of sheep X, this animal appearing to find the mixture of minerals and mineral-deficient hay entirely unpalatable. On December 22, after having been on the increased ration for 10 days, its food consumption fell suddenly to 307 gm. This loss of appetite continued to be manifested over the next four days, the mean daily food consumption for this interval being only 550 gm. At this stage, it was deemed necessary to modify the ration of sheep X, and from December 27 to January 4, it was given half the experimental ration in the morning and a ration of 750 gm. of seeds hay plus 50 gm. of linseed cake in the afternoon. The afternoon ration was consumed completely, whereas the morning ration was scarcely touched, an indication of the unpalatability, so far as this sheep at this stage was concerned, of the mixture of minerals and mineral-deficient herbage. Sheep X was brought back on to the experimental ration on January 5 and was in the metabolism crate from January 7 to January 27 for the fourth digestion trial. Its mean daily food consumption over this period was only 712 gm.

It cannot be said, therefore, that the addition of the mineral supplement had led to any improvement in the palatability of the mineral-deficient herbage. Indeed, what evidence there was would rather lead to the belief that the reverse was true. Although it is perhaps unwise to generalize on the basis of the results obtained in a single investigation with two animals, the evidence of the palatability tests certainly suggests the advisability of seeking to improve the mineral content of mineral-deficient pasturage by indirect methods, viz., by the application of suitable fertilizers to the pastures, rather than by the direct method of supplementing such deficient herbage with mineral mixtures.

After the completion of period 4, the ration of 1200 gm. of hay plus 30 gm. of minerals was enriched by the addition of 28 gm. of cod-liver oil, the object being to ascertain whether, in view of the deficiency of ether extract in the mineral-deficient hay, an increase in the amount of digestible oil in the ration would lead to an improvement in palatability. Actually, however, it was found that the addition of the oil depressed the appetites of the animals, the mean daily food consumption of sheep IX falling, during the first week, from 1109 to 896 gm., and that of sheep X from 739 to 681 gm. During the next four weeks, both sheep displayed a partial recovery of appetite, but their food consumption throughout remained well below the level which had been reached on the diets of hay and of hay plus minerals.

There is danger, therefore, that the addition of oils such as cod-liver

oil to the rations of farm animals may lead to diminished food consumption. This behaviour has also been noted in the case of dairy cows (13). Manifestly, however, it would be unsafe to stress this finding unduly, since farm animals, like human beings, do not react consistently to changes of diet. Moreover, if the period of feeding on the oil-containing diet had been prolonged sufficiently, the adverse effect of the cod-liver oil on palatability would probably have been found to disappear.

Influence of mineral supplement on the digestibility and nutritive value of the mineral-deficient herbage. An examination of the data in Table VI shows that the addition of the mineral mixture to the ration brought about no significant alteration, either improvement or depression, in the digestibility of the mineral-deficient hay. The first digestion trial of the mineral-supplemented hay (period 3) was begun after the sheep had been on the diet for 10 days. It will be noted that the mean digestion coefficient of the organic matter of the hay in this period (48·5 per cent.) was almost identical with the value (48·0 per cent.) obtained in period 2, when the mineral-deficient hay was fed alone. The differences between the mean digestion coefficients of the crude protein, N-free extractives and crude fibre were also small and of no significance, being of slighter magnitude than the differences observed between the values for the individual sheep in both periods 2 and 3 (see Appendix). There was an indication that the ether extract of the hay was digested somewhat more efficiently as a result of the addition of the mineral mixture, but this finding cannot be accepted with confidence, in view of the uncertainty attaching to measurements of the digestibility of the ether extract of oil-deficient fodders. This uncertainty is emphasized by the result obtained in period 4 (second digestion trial of hay plus minerals), when a negative digestion coefficient was obtained for the ether extract of the mineral-deficient hay.

The digestion measurements of the mineral-supplemented hay were repeated in period 4, when the sheep had been on the diet for 8 weeks. Again, having regard to the differences exhibited by the individual sheep in the different periods (see Appendix), it was to be concluded that the addition of the mineral supplement had exerted no significant influence on the extent to which the constituents of the mineral-deficient hay were digested.

The data in Table VII, showing the percentages of digestible nutrients and starch equivalent in the hay, calculated on the basis of the mean digestion coefficients obtained in the periods of feeding on hay alone and on hay plus minerals, reveal the fact that the addition of the mineral supplement did not increase, significantly, the amount of starch equiva-

lent, or net energy, which the sheep were able to derive from the mineral-deficient hay, the starch equivalents, per 100 lb. of dry matter, being 23·74 and 25·77 (periods 1 and 2 on hay alone) and 26·62 and 24·51 (periods 3 and 4 on hay plus minerals). The corresponding figures for the total digestible organic matter, per 100 lb. of the dry matter of the hay, were 43·86, 46·37, 46·87 and 45·55 lb. respectively. This finding, in respect of the absence of any significant effect on the nutritive value of the hay as a result of adding the mineral supplement, is in harmony with the conclusion enunciated in the previous section of this paper, namely, that the amount of net energy which ruminant animals are able to secure from any form of herbage is independent of the mineral content and depends solely on the amount and character of the organic constituents.

It has already been shown that the addition of cod-liver oil to the ration led to a decrease in the food consumption of the sheep. The results in Table VI demonstrate further that the inclusion of the oil did not bring about any improvement in the extent to which the hay was digested, but rather, if anything, caused a slight depression of digestibility. This effect was most noticeable in the case of the N-free extractives of the hay, the mean digestion coefficient of this constituent being the lowest recorded during the whole series of digestion measurements.

Referring again to Table V, it will be noted that over a period of 7 weeks (November 19 to January 6), sheep IX consumed sufficient of the ration of mineral-deficient hay plus mineral supplement to enable its live-weight to be maintained, a statement which also holds true of sheep X over the 5 weeks' period from November 19 to December 23. During these periods, the mean daily food consumption of sheep IX was 1172 gm. (= 1032 gm. of dry matter) and that of sheep X was 1189 gm. (= 1046 gm. of dry matter). During the final period of feeding on the ration of hay, minerals and cod-liver oil, sheep IX was again able to maintain its live-weight over the 5 weeks of experiment. In Table VIII are given the mean amounts of starch equivalent which were consumed daily by the sheep, per 100 lb. of live-weight, over those periods of the trial in which maintenance of live-weight was noted to have been established.

Table VIII. *Starch equivalent requirements for maintenance of live-weight in the sheep during the different periods of feeding.*

Period of feeding	Sheep IX lb. S.E. per 100 lb. L.-W.	Sheep X lb. S.E. per 100 lb. L.-W.
(1) Mineral-deficient hay alone	0·82	0·74
(2) Mineral-deficient hay + minerals	0·80	0·67
(3) Mineral-deficient hay + minerals + cod-liver oil	0·76	—

The results in Table VIII display a reasonable degree of uniformity for work on the food requirements of animals. As was pointed out in a previous section, the requirements of the sheep for maintenance were significantly lower than would have been anticipated on the basis of the recently-accepted standard of 1·26 lb. of starch equivalent per day per 100 lb. live-weight. The values obtained in the present work lay nearer the previously-accepted German standard, namely, 0·74 lb. of starch equivalent per 100 lb. live-weight.

It was only to be expected that, after subsisting for 30 weeks on a diet consisting mainly of forage, which, on account of its maturity, was of no better quality than meadow hay, the sheep would be in a backward condition from the standpoint of the butcher. Such proved to be the case at the completion of the trial on March 3. That the sheep, however, were merely in poor condition and not radically impaired in health is proved by the fact that, after a period of fattening on the University Farm, they sold for a satisfactory price on the market at the beginning of July. The most noteworthy observation respecting the condition of the sheep during the trial was the tendency of sheep X to become "pot-bellied." This tendency was first displayed in the period of feeding on the ration of 1500 gm. of mineral-deficient hay (September 10 to October 28). It was not so marked during the second digestion trial on mineral-deficient hay alone, but became very pronounced again during period 3 (hay plus minerals) and continued so up to December 22, when the sheep suddenly declined to consume the experimental ration (see Table V).

Nitrogen and mineral metabolism in the sheep.

The figures obtained in the measurements of the nitrogen and mineral balances do not permit of any conclusions being drawn respecting the utilization of these constituents by grazing animals on mineral-deficient pastures, since the experimental herbage, being at the hay stage of maturity, would presumably be poorer in chemical composition, both organic and inorganic, than the herbage as maintained in an immature condition by grazing. Further, it is probable that the food consumption of the animals, and consequently their mineral intake, would be greater when subsisting on the younger herbage under outdoor conditions than on the fibrous hay under conditions of indoor feeding. Nevertheless, the figures obtained in the present investigation are of interest in throwing light on certain aspects of mineral metabolism in animals on lime and phosphate-deficient diets, and in showing how the mineral metabolism may be modified by the addition of a mineral supplement to the deficient

ration. The complete data are recorded in the tables in the appendix to this communication.

Lime and phosphate metabolism. The essential data are given in Table IX. For fuller details the Appendix should be consulted.

Table IX. *Lime and phosphoric acid balances.*

		Lime (CaO)		Phosphoric acid (P_2O_5)	
Period	Sheep	Mean daily intake gm.	Mean daily balance gm.	Mean daily intake gm.	Mean daily balance gm.
1. Hay alone	IX	1·99	−2·40	1·44	−3·15
	X	2·86	−1·92	2·36	−1·91
2. Hay alone	IX	2·67	−1·10	2·88	−0·80
	X	2·65	−0·75	2·88	−0·59
3. Hay + minerals	IX	9·27	+1·50	4·47	−0·42
	X	11·31	+0·77	5·06	+0·46
4. Hay + minerals	IX	8·40	+1·43	4·71	+0·20
	X	6·42	+1·06	3·30	+0·59
5. Hay + minerals + cod-liver oil	IX	11·07	+0·33	4·92	−0·61
	X	8·55	+0·94	3·89	+0·60

Diverse results have been obtained by different workers in investigations into the lime and phosphate requirements for maintenance in adult sheep. This, however, is not surprising, since the rate of gain or loss of lime or phosphoric acid in the body is not governed solely by the amounts of these constituents in the ration, but also to a certain extent by the general mineral balance of the food. Thus, for example, a deficiency of phosphoric acid in the diet leads to a draft on the bones for this mineral. The process whereby phosphoric acid is liberated from the skeleton, however, necessitates the complete breakdown of the equivalent weight of bone ash. Not only phosphoric acid, therefore, but also lime, magnesia, soda, etc. find their way from the bones into the circulation, and if the concentrations of the latter in the blood are thereby caused to rise beyond the normal, the excesses are eliminated from the body. It has also been demonstrated that an excess of potash salts in the diet leads not only to increased potash elimination from the body, but also to an increased rate of loss of soda. If this condition continues long enough to necessitate the withdrawal of soda from the skeleton, then a corresponding withdrawal of lime, phosphoric acid, etc. also occurs, resulting in an increased rate of excretion of these minerals from the body.

In defining the mineral requirements for maintenance, therefore, account must be taken not only of the magnitude of the mineral supply, but also of the mineral balance of the food. For this reason, it is only to be expected that the experiments of different investigators, employing

rations of varying mineral balance, would yield discordant results. There can be no absolute figure representing, for example, the maintenance lime requirement, since the latter must vary with alterations of the mineral balance of the ration.

Kellner(14) states that full-grown sheep require only very small amounts of bone-forming substances, and that 2-year-old wethers, weighing 120 lb., had been found to need a daily supply of no more than 0·57 gm. of lime and 0·05 gm. of phosphoric acid. In the present investigation, however, while the sheep were on the ration of mineral-deficient hay (periods 1 and 2), the balances for both lime and phosphoric acid were negative throughout on rations containing from 1·99 to 2·86 gm. of lime and from 1·44 to 2·88 gm. of phosphoric acid. Taking the mean results for the 2 sheep in both periods, it is seen that on a daily ration supplying about 2·5 gm. of lime and 2·4 gm. of phosphoric acid, the mean daily balances in the sheep were – 1·55 gm. CaO and – 1·61 gm. P_2O_5. Since the mean daily intakes of lime and phosphoric acid, over the whole 15 weeks' period of feeding on mineral-deficient hay, were approximately 2·9 and 2·7 gm. respectively, it may be assumed that, on an average, the sheep were being depleted daily of something like $1\frac{1}{2}$ gm. each of lime and phosphoric acid. This represents, over the whole period, a total loss from the body of about 157 gm. of lime and the same amount of phosphoric acid, *i.e.*, in the neighbourhood of 300 gm. of tricalcic phosphate. That this was a serious loss is apparent, when it is remembered that a store sheep contains normally rather less than 800 gm. CaO and 700 gm. P_2O_5.

It is clear, therefore, that the mineral-deficient hay was unable to maintain lime and phosphate equilibrium in adult sheep. Such herbage, therefore, would fall far short of supplying the lime and phosphoric acid necessary for both maintenance and growth in younger animals. The mean results of periods 3 and 4, when the hay was supplemented with minerals, indicate that the sheep were able to store, per day, 1·19 gm. of lime and 0·21 gm. of phosphoric acid from a daily ration containing about 8·9 gm. of lime and 4·4 gm. of phosphoric acid, *i.e.*, on a ration containing, on the dry matter basis, 0·97 per cent. of lime and 0·48 per cent. of phosphoric acid.

The balance results of period 5 (see Table IX), though of an inconsistent nature in respect of the phosphate figures, do not reveal any improvement of lime assimilation as a result of adding cod-liver oil to the ration of hay plus minerals, although presumably at this stage the sheep were still in need of lime for re-building the skeletal reserves which had been depleted in the first period of feeding on mineral-deficient hay alone.

Potash, soda and chlorine metabolism. The results for the potash balances support the contention that farm animals are not likely to suffer from potash deficiency on any type of pasturage. In all 5 periods, with daily potash intakes ranging from 7·47 to 12·71 gm., positive potash balances of magnitudes varying from 0·16 to 2·39 gm. per day were obtained.

The data for the soda balances indicate that the requirement of adult sheep for this constituent is small. If the results for periods 1 and 2 (hay alone) be averaged, it appears that a mean daily intake of 1·47 gm. of soda was sufficient for a slow storage at the rate of about $\frac{1}{4}$ gm. per day. A rather surprising result was encountered in period 3, however, after the addition of the mineral supplement to the ration, since although the daily soda intake had risen to 2·32 gm. (sheep IX) and 2·68 gm. (sheep X), small negative balances were registered for both sheep, − 0·67 gm. in the case of sheep IX and − 0·23 gm. for sheep X. In periods 4 and 5, however, with daily intakes of soda varying from 1·29 to 2·44 gm., the balances were again slightly on the positive side of equilibrium.

It is of interest to note that the addition of the mineral supplement led to a large increase in the output of urinary potash from the sheep, with a corresponding decrease in the amount of faecal potash. In period 2 (hay alone), for example, about 33 per cent. of the total output of potash from sheep X was excreted in the urine, the remaining 67 per cent. appearing in the faeces. In period 3 (hay plus minerals), however, only 23 per cent. of the total potash excreted was lost in the faeces, whilst the proportion voided in the urine had risen to 77 per cent. Similar results were also noted in periods 4 and 5, and it must be concluded that the addition of the mineral supplement, containing calcium, phosphorus, sodium and chlorine, but no potassium, had radically affected the means whereby potash was eliminated from the bodies of the sheep.

The detailed results for the chlorine balances, as is only too common with short period balance measurements, are somewhat inconsistent and difficult of interpretation. If, however, the results for both animals in all five periods be averaged, it is seen that the adult wethers displayed neither tendency to lose nor to store chlorine, equilibrium, considering the experiment as a whole, being established almost exactly on a mean daily intake of about 4·5 gm. Cl. It will be noted that the main path of excretion of chlorine was through the urine. This is in sharp distinction to the manner of excretion of phosphoric acid, which was by way of the intestine.

Magnesia metabolism. The results for the magnesia balances, given

in the Appendix, show that the requirement of adult sheep for this mineral is quite small, and that full-grown animals display little or no tendency to store further magnesia in their bodies. In all the 5 periods, with mean daily intakes of magnesia ranging from 1·86 to 3·87 gm., the daily balance in every case denoted approximate magnesia equilibrium. Averaging the results for both sheep for all periods, the mean daily magnesia balance was + 0·08 gm. on a mean daily intake of 2·8 gm. MgO. It will be noted that in period 3, sheep X was in exact magnesia equilibrium on an intake of 2·88 gm. MgO per day. It is of interest to find that in periods 1 and 2, when the sheep were in both lime and phosphate deficit, no abnormal features were noted in respect of magnesia metabolism. It should also be pointed out that the main path of excretion of magnesia in the present trials was through the intestine into the faeces.

Nitrogen metabolism. In Table X are given the data respecting nitrogen intake and nitrogen balance throughout the trials. For fuller details the reader should consult the Appendix.

Table X. *Summary of nitrogen balances in the sheep.*

	Sheep IX		Sheep X	
Period	Mean daily intake gm. N	Mean daily balance gm. N	Mean daily intake gm. N	Mean daily balance gm. N
1. Hay alone	13·96	−3·45	20·52	+0·79
2. Hay alone	21·45	−0·82	21·43	+1·68
3. Hay + minerals	20·02	−0·73	21·30	+2·76
4. Hay + minerals	20·83	+0·28	13·68	+1·02
5. Hay + minerals + cod-liver oil	17·84	−1·01	13·45	+0·42

The figures in Table X illustrate the difficulty which frequently arises in interpreting the results of balance experiments. With the exception of period 4, on rations supplying from 13·96 to 21·45 gm. of nitrogen per day, sheep IX registered negative nitrogen balances, whereas sheep X, on daily intakes of nitrogen ranging from 13·45 to 21·43 gm., was in positive nitrogen balance throughout. The results do not necessarily indicate, however, that sheep IX was slowly losing flesh continuously over the whole period of the trial, since from September 17 to October 28, when the animals were receiving an increased ration of hay, the mean daily nitrogen intake of sheep IX was somewhat higher than that during the digestion periods, amounting to about 24 gm. per day. During this part of the trial, therefore, it may be assumed, on the basis of the results in periods 2, 3 and 4, that sheep IX was probably in nitrogenous equilibrium

or even storing small amounts of nitrogen in the form of flesh. This statement probably also holds true for the period of feeding on the increased ration of hay plus minerals (December 12 to January 6).

Nor must the results be taken as indicating that the herbage, under natural conditions of grazing, would be unable to meet the demands of growing animals for nitrogen. Even in its mature and fibrous form, it is moderately rich in protein, containing 13 per cent. on the basis of dry matter. It may be inferred, therefore, that such mineral-deficient herbage, if kept young and leafy by grazing, would have the high protein content which characterizes normal pasturage under such conditions.

The results do not, at first sight, suggest that there is any very close connection between protein and mineral metabolism in the animal. In periods 1 and 2, sheep X registered a positive nitrogen balance, but negative balances in respect of lime and phosphoric acid, whereas the balances for sheep IX were negative in every case. It would be unwise, however, to insist on this point too strongly, since the rather mysterious negative phosphate balances for sheep IX in periods 3 and 5, when minerals were included in the ration, might be capable of explanation if they could be correlated with the negative nitrogen balances for this sheep in these periods. It is certainly very suggestive that in period 4, when the nitrogen balance became slightly positive, a small positive phosphate balance was also recorded for sheep IX.

CONCLUSION.

In the consideration of the reason for the failure of grazing animals to thrive in regions of mineral-deficient pasturage, the standpoint may be taken that the failure may sometimes be due as much to the energy-deficient character of the herbage as to its deficiency in minerals. The starch value of such herbage may be so low that animals, even when consuming it to the limit of appetite, may not be able to secure sufficient net energy for normal development. The further question may also arise as to the method whereby mineral deficiencies cause the symptoms of malnutrition. Although it is clear that mineral deficiency must operate adversely by preventing normal development of the skeletal structure, it is possible that the following factors may also be contributory: (1) the digestibility of the organic constituents of the herbage may be depressed in the absence of an adequate supply of minerals; (2) the lack of sufficient mineral matter may confer an unpalatable character on the herbage, a

circumstance which may react adversely on the appetites of the grazing animals; (3) the physiological utilization of the digested nutrients may not proceed normally, if the amount of minerals in the diet is deficient.

In the investigation which has been described in the present communication, an attempt has been made to secure information which might furnish an answer to these and kindred questions. A detailed study has been made of the utilization by sheep of a sample of mineral-deficient herbage. The latter consisted mainly of purple melick grass and brown bent grass, with a small amount of sheep's fescue and traces of Yorkshire fog, sweet vernal grass, rough stalked meadow grass and fiorin. Clover and other leguminosae were not present, even in traces. From the chemical standpoint, the herbage was deficient in total ash, the most serious mineral deficiencies being in respect of lime and phosphoric acid. The supply of soda was also on the low side, whereas the percentages of potash, magnesia and chlorine were similar to the amounts of these constituents in "normal" herbage.

The following main conclusion has been drawn: The results lead to the belief that the failure of stock to thrive in mineral-deficient pastoral areas cannot be explained by assuming that the low mineral content is responsible for a lack of palatability in the herbage and a consequent depression of appetite in the grazing animals. Neither is there any evidence that the shortage of minerals causes the herbage to be digested any less efficiently than "normal" cultivated herbage of similar maturity and organic composition. The amount of net energy which ruminant animals are able to derive from any form of herbage seems to be independent of the mineral content and to depend solely on the amount and character of the organic constituents.

Malnutrition on pasturage of sub-normal mineral content is due directly to the failure of the diet to supply the necessary inorganic materials for constructional purposes and for maintaining the normal balance of minerals in the blood and tissues, and is not, even in part, to be ascribed to any indirect effect, such as is embodied in the suggestion that the mineral deficiency leads to under-nutrition of the animal by causing a depression of its appetite and its capacity to digest the organic constituents of the herbage.

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(*Received September 1st*, 1930.)

APPENDIX.

I. Digestion Tables.

	Sheep IX							Sheep X						
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Period 1 (hay alone)														
Food supply	1041·00	1003·83	134·60	22·90	488·54	357·79	37·17	1041·00	1003·83	134·60	22·90	488·54	357·79	37·17
Food residues	366·60	352·78	47·40	6·86	173·58	124·94	13·82	50·70	48·06	6·41	1·32	23·39	16·94	2·64
Food intake	674·40	651·05	87·20	16·04	314·96	232·85	23·35	990·30	955·77	128·19	21·58	465·15	340·85	34·53
Voided	371·20	349·37	44·06	13·62	190·28	101·41	21·83	558·70	529·37	64·70	20·67	288·35	155·65	29·33
Digested	303·20	301·68	43·14	2·42	124·68	131·44	1·52	431·60	426·40	63·49	0·91	176·80	185·20	5·20
Digestion coefficients, %	44·96	46·34	49·47	15·09	39·59	56·45	6·51	43·58	44·61	49·53	4·22	38·01	54·33	15·06
Period 2 (hay alone)														
Food supply	1045·70	1010·15	135·94	19·87	488·55	365·79	35·55	1045·70	1010·15	135·94	19·87	488·55	365·79	35·55
Food residues	14·60	13·53	1·84	0·21	6·75	4·73	1·07	15·30	14·09	2·00	0·35	7·13	4·61	1·21
Food intake	1031·10	996·62	134·10	19·66	481·80	361·06	34·48	1030·40	996·06	133·94	19·52	481·42	361·18	34·34
Voided	559·60	531·67	67·15	17·96	282·26	164·30	27·93	530·70	503·69	61·46	18·95	277·82	145·46	27·01
Digested	471·50	464·95	66·95	1·70	199·54	196·76	6·55	499·70	492·37	72·48	0·57	203·60	215·72	7·33
Digestion coefficients, %	45·73	46·65	49·93	8·65	41·42	54·49	19·00	48·50	49·43	54·11	2·92	42·29	59·73	21·34
Period 3 (hay +minerals)														
Food supply	1085·80	1020·20	134·75	21·12	495·47	368·86	65·60	1085·80	1020·20	134·75	21·12	495·47	368·86	65·60
Food residues	91·60	72·66	9·65	1·24	35·77	26·00	18·94	25·60	12·38	1·61	0·23	2·86	7·68	13·22
Food intake	994·20	947·54	125·10	19·88	459·70	342·86	46·66	1060·20	1007·82	133·14	20·89	492·61	361·18	52·38
Voided	542·17	508·71	57·63	14·57	282·37	154·14	33·46	533·33	497·43	60·38	16·72	280·47	139·86	35·90
Digested	452·03	438·83	67·47	5·31	177·33	188·72	13·20	526·87	510·39	72·76	4·17	212·14	221·32	16·48
Digestion coefficients, %	45·47	46·31	53·93	26·71	38·58	55·04	28·29	49·70	50·64	54·65	20·00	43·06	61·27	31·46
Period 4 (hay +minerals)														
Food supply	1091·70	1024·30	141·99	18·27	486·92	377·12	67·40	701·80*	658·47	91·28	11·74	313·02	242·43	43·33
Food residues	107·20	86·49	11·83	1·57	43·55	29·54	20·71	51·80	41·32	5·67	0·81	20·56	14·28	10·48
Food intake	984·50	937·81	130·16	16·70	443·37	347·58	46·69	650·00	617·15	85·61	10·93	292·46	228·15	32·85
Voided	547·75	520·43	57·88	18·45	289·52	154·58	27·32	328·37	309·81	38·25	11·49	175·01	85·06	18·56
Digested	436·75	417·38	72·28	—	153·85	193·00	19·37	321·63	307·34	47·36	—	117·45	143·09	14·29
Digestion coefficients, %	44·36	44·50	55·53	—	34·70	55·52	41·49	49·48	49·80	55·32	—	40·16	62·71	43·50
Period 5 (hay +minerals +cod-liver oil)														
Hay	905·14	869·48	117·22	15·30	412·01	324·95	35·66	678·86	652·11	87·91	11·47	309·02	243·71	26·75
Minerals†	25·48	—	—	—	—	—	25·48	19·11	—	—	—	—	—	19·11
Cod-liver oil	23·99	23·99	—	23·99	—	—	—	18·26	18·26	—	18·26	—	—	—
Total food supply	954·61*	893·47	117·22	39·29	412·01	324·95	61·14	716·23*	670·37	87·91	29·73	309·02	243·71	45·86
Food residues	51·57	41·87	5·69	2·60	20·10	13·48	9·70	35·64	29·43	3·87	1·90	14·09	9·57	6·21
Food intake	903·04	851·60	111·53	36·69	391·91	311·47	51·44	680·59	640·94	84·04	27·83	294·93	234·14	39·65
Voided	510·17	478·85	53·98	14·69	268·30	141·88	31·32	353·19	331·61	41·22	10·35	185·67	94·37	21·58
Digested	392·87	372·75	57·55	22·00	123·61	169·59	20·12	327·40	309·33	42·82	17·48	109·26	139·77	18·07
Digestion coefficients, %	43·51	43·77‡	51·60	59·95‡	31·54	54·45	39·11	48·10	48·26‡	50·95	62·81‡	37·04	59·69	45·57

* Food residues were not allowed to accumulate in these periods, but were fed on certain days instead of usual ration.

† In the periods when the minerals were fed, the figures for mineral supply include the carbon dioxide in the calcium carbonate of the supplement.

‡ Assuming cod-liver oil to be digested completely, the digestion coefficients of the organic matter of the hay were 42·14 per cent. (sheep IX) and 46·74 per cent. (sheep X). Mean = 44·4 per cent. Digestion coefficients of ether extract are negative.

II. Nitrogen and Mineral Balances.

	Sheep IX							Sheep X						
				Voided							Voided			
	Supply gm.	Residues gm.	Net intake gm.	Faeces gm.	Urine gm.	Total gm.	Mean daily balance gm.	Supply gm.	Residues gm.	Net intake gm.	Faeces gm.	Urine gm.	Total gm.	Mean daily balance gm.
Period 1 (hay alone)														
N	21·54	7·58	13·96	7·05	10·36	17·41	−3·45	21·54	1·02	20·52	10·35	9·38	19·73	+0·79
CaO	3·02	1·03	1·99	3·16	1·23	4·39	−2·40	3·02	0·16	2·86	4·02	0·76	4·78	−1·92
P_2O_5	2·50	1·06	1·44	4·57	0·02	4·59	−3·15	2·50	0·14	2·36	4·25	0·02	4·27	−1·91
K_2O	11·76	4·29	7·47	5·31	2·00	7·31	+0·16	11·76	0·56	11·20	8·32	1·67	9·99	+1·21
Na_2O	2·08	0·48	1·60	1·04	0·22	1·26	+0·34	2·08	0·04	2·04	1·01	0·06	1·07	+0·97
MgO	4·06	1·25	2·81	2·08	0·50	2·58	+0·23	4·06	0·19	3·87	3·41	0·62	4·03	−0·16
Cl	4·58	1·47	3·11	0·07	2·45	2·52	+0·59	4·58	0·19	4·39	0·39	4·00	4·39	0·00
Period 2 (hay alone)														
N	21·75	0·30	21·45	10·74	11·53	22·27	−0·82	21·75	0·32	21·43	9·83	9·92	19·75	+1·68
CaO	2·72	0·05	2·67	3·19	0·58	3·77	−1·10	2·72	0·07	2·65	3·14	0·26	3·40	−0·75
P_2O_5	2·93	0·05	2·88	3·64	0·04	3·68	−0·80	2·93	0·05	2·88	3·45	0·02	3·47	−0·59
K_2O	12·34	0·17	12·17	6·88	4·34	11·22	+0·95	12·34	0·15	12·19	6·79	3·31	10·10	+2·09
Na_2O	1·15	0·03	1·12	1·45	0·10	1·55	−0·43	1·15	0·02	1·13	1·01	0·05	1·06	+0·07
MgO	3·24	0·06	3·18	1·96	0·75	2·71	+0·47	3·24	0·05	3·19	2·12	0·47	2·59	+0·60
Cl	4·71	0·08	4·63	0·28	6·06	6·34	−1·71	4·71	0·05	4·66	0·48	4·23	4·71	−0·05
Period 3 (hay + minerals)														
N	21·56	1·54	20·02	9·22	11·53	20·75	−0·73	21·56	0·26	21·30	9·66	8·88	18·54	+2·76
CaO	16·80	7·53	9·27	7·39	0·38	7·77	+1·50	16·80	5·49	11·31	10·15	0·39	10·54	+0·77
P_2O_5	6·91	2·44	4·47	4·85	0·04	4·89	−0·42	6·91	1·85	5·06	4·58	0·02	4·60	+0·46
K_2O	12·88	0·93	11·95	2·61	7·72	10·33	+1·62	12·88	0·17	12·71	2·39	8·03	10·42	+2·29
Na_2O	4·00	1·68	2·32	2·60	0·39	2·99	−0·67	4·00	1·32	2·68	2·63	0·28	2·91	−0·23
MgO	3·06	0·38	2·68	2·43	0·42	2·85	−0·17	3·06	0·18	2·88	2·47	0·41	2·88	0·00
Cl	7·10	1·16	5·94	0·23	5·31	5·54	+0·40	7·10	0·78	6·32	0·38	5·27	5·65	+0·67
Period 4 (hay + minerals)														
N	22·72	1·89	20·83	9·26	11·29	20·55	+0·28	14·59	0·91	13·68	6·12	6·54	12·66	+1·02
CaO	16·17	7·77	8·40	6·52	0·45	6·97	+1·43	10·39	3·97	6·42	5·16	0·20	5·36	+1·06
P_2O_5	7·37	2·66	4·71	4·49	0·02	4·51	+0·20	4·74	1·44	3·30	2·69	0·02	2·71	+0·59
K_2O	12·53	1·10	11·43	3·34	7·12	10·46	+0·97	8·06	0·55	7·51	1·51	3·61	5·12	+2·39
Na_2O	4·11	1·67	2·44	1·64	0·44	2·08	+0·36	2·64	0·93	1·71	0·99	0·36	1·35	+0·36
MgO	3·19	0·44	2·75	2·30	0·48	2·78	−0·03	2·05	0·19	1·86	1·81	0·20	2·01	−0·15
Cl	7·33	2·25	5·08	0·05	4·82	4·87	+0·21	4·72	1·05	3·67	0·06	3·48	3·54	+0·13
Period 5 (hay + minerals + cod-liver oil)														
N	18·75	0·91	17·84	8·64	10·21	18·85	−1·01	14·07	0·62	13·45	6·60	6·43	13·03	+0·42
CaO	13·84	2·77	11·07	10·37	0·37	10·74	+0·33	10·38	1·83	8·55	7·28	0·33	7·61	+0·94
P_2O_5	6·12	1·20	4·92	5·51	0·02	5·53	−0·61	4·60	0·71	3·89	3·27	0·02	3·29	+0·60
K_2O	11·13	0·53	10·60	2·49	7·73	10·22	+0·38	8·35	0·37	7·98	1·52	4·78	6·30	+1·68
Na_2O	3·15	1·76	1·39	1·24	0·13	1·37	+0·02	2·36	1·07	1·29	0·86	0·34	1·20	+0·09
MgO	2·62	0·16	2·46	2·11	0·40	2·51	−0·05	1·97	0·10	1·87	1·66	0·19	1·85	+0·02
Cl	6·34	2·22	4·12	0·23	4·64	4·87	−0·75	4·76	1·25	3·51	0·08	3·22	3·30	+0·21

THE DEVELOPMENT OF THE SOIL PROFILE IN NORTH WALES AS ILLUSTRATED BY THE CHARACTER OF THE CLAY FRACTION.

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Introductory.

The study of the soil profiles of an area may be expected to throw light on the pedogenic processes which have operated in the past and to afford some indication of the tendencies to change under contemporary conditions. North Wales, with its large proportion of uncultivated land, offers abundant opportunities for the study of soil profiles which have not been disturbed by recent tillage operations. In the present paper it is proposed to consider some typical profiles, with special reference to the variations in composition of the clay fraction in the different horizons. Before dealing with actual profiles, however, it will be helpful to summarise briefly the principal features of the topography, climate, and geology of our area.

Characteristics of the area.

North Wales is generally hilly or mountainous, rising in Snowdon to 3571 feet above sea level. Its mountainous character is the more marked because the highest mountains are no more than a few miles from the sea coast. Apart from certain estuarine alluvial flats and comparatively narrow valley bottoms, there is very little level ground. The lowlands have generally a rolling topography.

The climate is typically maritime. In the coastal areas the mean January temperature is 5–6° C., and the mean July temperature about 15–16° C., the winter being often almost frost-free and the snowfall negligible. Wider variations between summer and winter temperatures are found towards the east, with rather more severe winters and hotter summers. There is also, of course, a fall in temperature with altitude, so that the mountains are frequently covered with snow during the winter and early spring months. The mean annual rainfall, which is fairly well distributed without any well-defined wet or dry periods, varies from 25 in. in certain coastal lowlands to nearly 200 in. below the peak of Snowdon. It may be estimated that about 73 per cent. of the area has a mean annual rainfall greater than 35 in., 43 per cent. greater than 40 in., 17 per cent. greater

than 50 in., and 5 per cent. greater than 100 in. The prevailing winds are from the south-west, and the atmosphere is generally very humid.

The character of the temperature conditions relative to other localities may be judged from the following figures for mean annual temperature calculated from the meteorological data for 1926, 1927 and 1928, collected by the Ministry of Agriculture. The station at Aber is 50 feet above sea level.

Table I. *Mean annual temperatures in ° F. at certain British stations.*

Rothamsted	Wye	Long Ashton, Bristol	Newton Abbot, Devon	Penzance	Aber, Bangor
47·8	49·1	49·7	50·5	51·9	50·1

The mildness of the winter is evident from the following figures calculated for the mean temperature of the months of December, January, and February for the years 1926–8.

Table II. *Mean winter temperatures in ° F. at certain British stations.*

Rothamsted	Wye	Long Ashton, Bristol	Newton Abbot, Devon	Penzance	Aber, Bangor
39·3	40·5	43·6	44·2	46·2	44·7

The actual number of days in an average winter when the soil is frozen is small, and in some winters practically no freezing occurs at all.

The dominant feature of the climate is its extreme humidity. Among the factors proposed for expressing quantitatively the humidity of a given climate, two may be selected. R. Lang(1) has classified climates according to the "Rain Factor," *i.e.* the rainfall in mm. divided by the mean annual temperature in ° C. Taking 1000 mm. as the average annual rainfall of the lowlands of North Wales and 10° C. as the mean annual temperature we obtain the rain factor 100, a high figure which is greatly exceeded in the wetter uplands.

Another expression for humidity, which seeks to give a measure of the degree of leaching to which a soil is submitted, is R. Meyer's $N - S$ quotient(2). This is equal to $R \div T \times (100 - H)/100$, where R = rainfall in mm., T = tension of aqueous vapour at the mean temperature of the locality, and H = average humidity. In the case of Aber, Bangor, we have for the mean of 1926, 1927 and 1928: rainfall, 1152 mm.; mean humidity, 80·3 per cent.; mean temperature, 50·1° F., at which the tension of aqueous vapour is 9·2 mm. Hence, the $N - S$ quotient $= 1152/9{\cdot}2 \times 0{\cdot}197 = 636$. Comparison with other localities is given in the following table.

Table III. N – S *quotient for certain British stations.*

Rothamsted	Wye	Long Ashton, Bristol	Newton Abbot, Devon	Penzance	Aber, Bangor
433	457	573	500	606	636

The figure for Aber represents an extremely humid climate, favouring a podsolic type of profile development.

Geologically the area consists mainly of Pre-Cambrian, Cambrian, Ordovician and Silurian rocks with their associated drifts. There is a certain extent of Carboniferous rocks and a small amount of Trias which, however, occurs mainly as drift. It is to be noted that, with the exception of the Carboniferous Limestone, the rocks of the area are relatively poor in lime.

Comparatively little of the area is under its original vegetation. It is supposed that in primitive times the area was mainly under deciduous forest up to about 1700 feet. Much of the lowland was swamp or marsh. The deforestation of the area doubtless led to considerable erosion, as evidenced by the prevalence of hillwash, and it is probable that large areas which are now thin gravelly soil or bare rock were once under a soil cover in which, doubtless, a profile would have been discernible.

With the above climatic conditions, it might be expected that the mature profile would be of a podsolic type. The absence of primitive coniferous forest would exclude the occurrence of extreme podsols, and it seems likely that the mature profile over most of the lowlands of North Wales would approximate to the brown-earth type. Actually, although a large proportion of the area is uncultivated, deep, well-developed profiles are by no means common. This is due to a number of circumstances. In the first place, the prevalence of a hilly or rolling topography militates against profile development, for erosion tends to mask the effects of eluviation and may, indeed, result in the occurrence of truncated profiles. Secondly, sandy soils in which profiles are most readily developed are comparatively uncommon, except in the coastal dune areas, where, of course, wind erosion prevents good profile development. Texturally, our soils are generally stony silty loams which are naturally more resistant to the effects of leaching than sandy soils. Further, the rocks in North Wales are relatively hard and resistant to weathering. Consequently, in uplands, where waste lands occur, deep profiles are not to be expected. Indeed, even where profiles are found, the *B* horizon extends generally into the parent rock, so that it is not possible to distinguish between the *B* and *C* horizons.

Significance of the clay fraction.

The processes which result in the development of a soil profile affect both the soil organic matter and also the mineral matter. So far as the mineral profile is concerned, differences in the several horizons may be due to (1) mechanical eluviation resulting in removal of the finer fractions from the upper horizons and deposition in the lower horizons; (2) changes consequent on decomposition of the clay complex and differential movements of the principal constituents, mainly silica and sesquioxides; and (3) movements of the soluble salts including calcium carbonate, calcium sulphate and alkali salts. Podsolic and lateritic profiles result proximately from processes of the second category. Thus, in the podsol profile, the superficial layer is impoverished in sesquioxides, which are deposited in the *B* horizons. In the laterites and lateritic soils, the surface horizons are characterised by impoverishment in silica and relative enrichment in sesquioxides. Since these changes affect mainly the colloidal weathering complex, it is obvious that total analysis, in which the unweathered minerals are reckoned in with the weathering complex, can only give an approximate picture of the processes which have resulted in profile development. The differences in gross composition between the different horizons may be simply due to mechanical eluviation which has resulted in the translocation of one group of constituents.

Thus, whilst an examination of the character of the clay complex cannot give a complete definition of the soil profile, it may be expected to give a closer insight into the changes to which the more reactive portion of the soil has been subjected than is given by bulk analyses.

In a recent work on laterite, Harrassowitz (3) has submitted profile studies in which attention is focussed on the clay constituent by the use of acid extraction methods. Briefly the distinction drawn is that proposed by Van Bemmelen (4), depending on the different attack of hydrochloric and sulphuric acids respectively. The material dissolved by hydrochloric acid is regarded as the gel-like allophanoid weathering complex, whilst that which only yields to sulphuric acid is regarded as the kaolinitic weathering complex, considered to have originated principally from the weathering of the alkali felspars. The expression of profile characters in terms of the weathering complex rather than in terms of the total soil, including the unweathered minerals present, is certainly an advance. But it may be doubted if any method of acid extraction can be devised which only attacks the weathering complex and leaves the unweathered minerals unattacked. The present writer found that a powdered

quartz diorite yielded 10·8 per cent. of silica and 20·6 per cent. of sesquioxides on digestion with 20 per cent. hydrochloric acid for 48 hours at 100° C.

In an earlier communication, the writer (5) presented some preliminary results of investigations on the composition of the clay fraction, and suggested that this line of attack might be expected to yield valuable information on the nature of the clay complex. If it were possible to secure a rigid separation of the clay constituent or weathering complex from the unweathered mineral silicates of the soil, the variations in composition of this fraction should give a clear insight into the nature of the leaching processes which prevail in any given profile. In selecting the fraction having a settling velocity less than 0·0001 cm./sec., *i.e.* the clay according to the former English scale, the writer is aware that such a rigid separation cannot be assumed, and it is necessary to examine the limitations of the assumption made.

In the first place, it has been shown that the residue after removal of the clay fraction consists practically entirely of crystalline fragments. In connection with a recent co-operative study of mechanical analysis (6), representative samples of such residues were examined at the laboratory of the U.S. Bureau of Soils and found to contain only trifling percentages of colloidal matter. In the case of lateritic soils, which, however, do not fall within the scope of the present paper, it may be that material belonging properly to the weathering complex will also occur among the coarser fractions. Even here, however, the clay isolated by mechanical analysis may be no less truly representative of the weathering complex.

There is also the possibility that fragments of unweathered minerals may occur in the clay fraction. In some experiments made by the writer (7), the form of the mechanical composition curves of mechanically comminuted materials suggested a lower size limit corresponding with $\log V = \bar{5}{\cdot}0$, and there is thus the possibility that over the range $\log V = \bar{5}{\cdot}0$ to $\log V = \bar{4}{\cdot}0$ some unweathered mineral material may be present. Bradfield (8) found little difference in composition between the suspension fraction ($1{\cdot}2\mu - 0{\cdot}4\mu$) and the emulsoid fraction ($< 0{\cdot}1\mu$) of a subsoil. Joseph (9) considers the whole of the material of diameter less than 2μ colloidal.

There would thus appear to be a *prima facie* case for regarding the fraction $< 2\mu$ in diameter as generally equivalent to the inorganic colloidal weathering complex. In the present work the separation has been effected by the use of a settling velocity of 0·0001 cm./sec. rather than 10 cm./8 hours as used in the International method. This was used

because a large number of analyses had already been accumulated on this basis. It is unlikely that the use of the present International settling time would give appreciably different results. However open to criticism may be the assumption that the clay fraction represents the reactive weathering complex, it is, at any rate, reasonable to assume that variations in its composition in the soil profile do actually represent variations in the inorganic colloidal constituent and not segregations of unweathered mineral constituents. Indeed, it is difficult to see how they could be otherwise interpreted.

Before proceeding to the presentation and discussion of the results, it is desirable to outline the methods used. Since the complete dispersion of a soil with hydrogen peroxide and hydrochloric acid involves the solution of sesquioxides and, to a less extent, silica, from the clay, it was decided to use direct dispersion with ammonia. The soils were shaken for 24 hours with 1 per cent. ammonia and the clay fraction was then decanted off in the usual way. It was not possible, in the case of highly organic soils, to remove all the clay by this method, but enough was collected for the purposes of analysis, it being assumed that the clay remaining unseparated would be of similar composition. The united pourings off were evaporated to dryness, ignited, and ground to pass a 100-mesh sieve. Silica, ferric oxide, alumina and titanium oxide were determined after fusion with fusion mixture. The titanium oxide, which rarely exceeded 1 per cent. in amount, was determined in order to correct the figures for alumina and is not reported in the ensuing tables.

PROFILES WITH FREE DRAINAGE.

Uncultivated soils.

Proceeding to the description of some actual profiles, the first to be discussed are some in which the expected podsolic character is clearly marked by the field characteristics. The best profiles are naturally found on the soils with the most open texture and the smallest resistance to leaching.

Esclusham, near Wrexham, Denbighshire. Altitude: 1000 ft. Rainfall: 45 in. Parent material: Millstone Grit. Vegetation: *Calluna vulgaris*, *Festuca* spp.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
A_0	0–9	Peaty layer	40·7	3·28
A_1	9–15	Grey sandy loam	5·9	3·24
B_2	15–21	Reddish brown light loam	5·2	3·76
B–C	>21	Lighter brown rubbly light loam	1·8	4·14

Table IV. *Composition of clay fraction of successive layers of Esclusham profile (unignited material).*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3*	SiO_2/R_2O_3*
A_1	46·6	31·2	2·5	2·54	2·42
B	37·7	27·9	13·9	2·30	1·75
B–C	34·7	25·6	13·4	2·30	1·72

* Molecular ratios throughout.

It will be noticed that the differentiation affects primarily the ferric oxide, for the silica-alumina ratio does not change markedly with depth. The slight difference between the *B* and *B–C* horizons suggests that the latter belongs to the *B* horizon, although it is mingled with disintegrating rock. This is a frequent circumstance with Welsh upland profiles. In some cases, for example on a quartzite in Anglesey, the bleached *A* horizon passes directly into the parent rock, in the fissures of which brown staining occurs.

Cefn y Bryn, West Glamorganshire. Altitude: 300 ft. Rainfall: 40 in. Parent material: Old Red Sandstone conglomerate. Vegetation: *Ulex Gallii, Ulex Europaeus, Festuca* spp.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
A_0	0–4	Fibrous peat layer	—	—
A_1	4–7	Greyish sand	1·6	4·64
B	7–12	Reddish brown sandy loam	2·1	4·68
C	14–16	Yellowish brown sandy loam on rock	0·5	5·52

Table V. *Composition of clay fraction of successive layers of Cefn y Bryn profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
A_1	60·0	27·6	4·8	3·70	3·33
B	43·0	30·0	21·7	2·44	1·67
C	45·6	28·0	16·0	2·80	2·03

The figures suggest that, in this case, we are dealing definitely with *A*, *B*, and *C* horizons, and this is supported by the evidence in the field. Here, again, the eluviation affects principally the ferric oxide. The organic carbon figures suggest a slight accumulation of humic matter in the *B* horizon—an unusual circumstance in Welsh profiles.

Cwm Du, Beddgelert, Caernarvonshire. Altitude: 600 ft. Rainfall: 100 in. Parent material: hillwash from Ordovician shale. Vegetation: *Nardus stricta, Molinia coerulea, Festuca ovina, Juncus squarrosus, Vaccinium myrtillus, Calluna vulgaris,* Mosses.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
A	0–6	Dark greyish peaty loam	23·6	4·10
B	6–10	Reddish brown loam	5·6	4·22
C	10–20	Greyish shaly loam	3·4	4·54

Table VI. *Composition of clay fraction of successive layers of Cwm Du profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
A	43·5	24·7	13·2	3·00	2·55
B	35·7	32·6	24·8	1·86	1·25
C	41·4	30·4	28·4	2·31	1·45

Eluviation here affects principally the ferric oxide but also, in some degree, the alumina. The lowest horizon is probably to some extent of *B* character, as shown by the low silica-sesquioxide ratio.

Aber, Caernarvonshire. Altitude: 900 ft. Rainfall: about 60 in. Parent material: Ordovician shale. Vegetation: *Calluna vulgaris, Vaccinium myrtillus, Festuca* spp., Lichens.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
A_0	0–5	Dark peaty layer	13·8	3·64
A_1	·5–8	Grey stony loam	6·2	4·10
B_1	8–14	Reddish brown loam	4·7	4·68
B_2	14–20	Light brown shaly loam	3·5	4·70
B_3	20–26	Light brown very shaly loam	3·6	4·82

Table VII. *Composition of clay fraction of successive layers of Aber podsol profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
A_0	51·7	19·9	7·5	4·40	3·56
A_1	45·4	20·1	13·1	3·84	2·75
B_1	35·4	31·7	22·4	1·90	1·31
B_2	34·7	28·3	22·3	2·09	1·39
B_3	33·6	36·6	19·2	1·56	1·17

The figures for this profile show that in the lowest layer, below which it was impossible to sample on account of rock, the *C* horizon has not been reached. Indeed, the *B* character is most strongly marked in the material mixed with the disintegrating rock. The eluviation of the ferric oxide is more marked than that of the alumina.

The profiles hitherto described occur generally on the poorer types of waste land with a definitely heath type of vegetation. The figures for the *p*H show that the reaction is always extremely acid. From the point of view of grazing, the grass heaths, which generally occur below the heaths and above the limits of the cultivated land, are of a better character. The corresponding soil profiles do not show the marked differentiation into *A* and *B* horizons, and generally consist of a dark brown turfy layer changing to a lighter brown at 6–10 in. Rock is generally encountered at 12–24 in., except on hillwash and glacial drift. Typical grass heath profiles will now be described.

Dolwgan, near Criccieth, Caernarvonshire. Altitude: 800 ft. Rainfall: 75 in. Parent material: Ordovician shale drift. Grass vegetation.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–3	Turfy layer	10·3	4·7
II	3–6	Brown loam	5·0	4·5
III	6–9	Brown loam	2·5	5·1
IV	9–12	Light brown loam	1·7	5·0
V	12–15	Light brown shaly loam	1·1	5·0
VI	15–18	Light brown shaly loam with rock	2·0	4·8

Table VIII. *Composition of clay fraction of successive layers of Dolwgan profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	45·8	30·1	14·2	2·56	1·98
II	45·6	35·2	13·3	2·20	1·78
III	43·7	29·9	14·0	2·50	1·90
IV	43·3	34·8	15·2	2·11	1·65
V	44·7	38·8	16·7	2·18	1·65
VI	43·5	34·7	15·3	2·13	1·66

It will be seen at once that the differentiation is less marked than in the heath profiles. The field examination does not reveal the presence of a bleached layer, and there is no immediate evidence for assigning any part of the profile to an *A* or *B* horizon. Consideration of the figures does, however, suggest that we are dealing throughout with material of a *B* character in which some leaching of the sesquioxides is taking place, and which may eventually result in a bleached surface layer. The reason for the higher silica-alumina and silica-sesquioxide ratios in the third layer is not apparent.

Aber, near Bangor. Altitude: 800 ft. Rainfall: about 60 in. Parent material: Ordovician shale. Grass and bracken vegetation.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–3	Turfy layer	15·9	4·20
II	3–6	Dark brown turfy loam	8·1	4·42
III	6–9	Brown loam	5·4	4·58
IV	9–12	Light brown loam		4·78
V	12–15	Reddish brown shaly loam with rock	3·4	4·82

Table IX. *Composition of clay fraction of successive layers of Aber (grass heath) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	43·5	34·0	17·6	2·17	1·63
II	42·7	32·2	17·0	2·25	1·69
III	42·6	33·4	17·6	2·17	1·62
IV	41·1	35·8	18·0	1·95	1·48
V	39·0	31·9	20·6	2·12	1·50

Here, again, there is no well-marked differentiation. Comparison with the figures for the podsol profile, which is in the immediate vicinity, suggests that the whole of this profile has the character of *B* horizon material, for its high sesquioxide content is shown even in the top layer.

Bryniau Bach, near Bangor. Altitude: 250 ft. Rainfall: 40 in. Parent material: drift from volcanic ash. Vegetation: *Anthoxanthum odoratum, Deschampsia flexuosa, Agrostis alba, Luzula compestris.*

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–12	Brown turfy loam	7·7	4·86
II	12–20	Light brown stony loam	4·0	5·20
III	20–28	Orange brown loam	1·6	5·19
IV	> 28	Yellowish brown loam	1·5	4·92

Table X. *Composition of clay fraction of successive layers of Bryniau Bach profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	49·4	31·5	17·0	2·66	1·98
II	39·6	33·7	18·8	2·00	1·48
III	40·1	35·4	19·0	1·92	1·44
IV	42·0	36·3	17·0	1·97	1·51

Here there is rather more difference between the surface soil and the deeper layers, but there is not as yet any sign of bleaching.

The grass heath profiles are generally less acid than the podsol profiles of the heath soils. It will be noticed also that there is not such a marked accumulation of organic matter in the top layer as in the podsol profiles. There is no evidence of bleaching in the field, but the figures for the composition of the clay fraction suggest incipient podsolisation of the surface soil. The layers below the immediate surface show an excess of sesquioxides, and suggest *B* horizon material which, in an earlier stage, may well have extended to the surface. It is reasonable to suppose that, in time, the incipient podsolisation revealed in the superficial layer will become more accentuated and result in the development of a bleached layer. It is conjectured that this has actually occurred in the profiles under heath. If this diagnosis is correct, such profiles are really secondary in character.

Cultivated soils.

In the cultivated soils the natural profile has, of course, been disturbed by human interference, but the disturbance will not have extended to a greater depth than 9–12 in., and the data obtained will thus not be without significance. In North Wales, it is practically impossible to find soils under continuous arable cultivation. The general practice

is to cultivate for three years under oats, roots, and oats (or barley), and then to lay the land down to grass for a period of five years or more. It will be convenient to consider the profiles in two main classes, namely:

(i) Shallow sedentary soils with free drainage.

(ii) Deep drift or hillwash soils with free drainage.

Sedentary soils. Three examples of the first class will be considered.

Cefn, Beaumaris, Anglesey. Altitude: 300 ft. Rainfall: 36 in. Parent material: Gwna green schist (Pre-Cambrian). Anglesey soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–9	Brown loam	4·25	5·2
II	9–15	Light brown stony loam on rock	2·1	5·8

Table XI. *Composition of clay fraction of successive layers of Cefn profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	46·5	30·0	14·5	2·64	2·01
II	43·0	33·5	14·8	2·19	1·71

Although there is no field evidence by bleaching, a definite eluviation of the sesquioxides is indicated by the figures for the composition of the clay fraction.

Brithdir, near Bangor. Altitude: 320 ft. Rainfall: 40 in. Parent material: Ordovician quartz felsite. Bangor soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–8	Brown light loam	5·1	5·52
II	8–16	Reddish brown light loam	3·2	6·11

Table XII. *Composition of clay fraction of successive layers of Brithdir profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	40·5	28·0	11·2	2·46	1·95
II	41·1	36·1	10·4	1·93	1·63

The general picture is similar to that presented by the Cefn profile. From the point of view of soil survey, there seems little justification for keeping the Bangor and Anglesey series separate, so closely do their general field and laboratory characteristics approach each other.

Tyddyn Llwydyn, near Bangor, Caernarvonshire. Altitude: 150 ft. Rainfall: 40 in. Parent material: Ordovician flags. Powys soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–8	Buff brown shaly loam	5·2	5·68
II	8–15	Brown shaly loam on rock	1·9	6·22

Table XIII. *Composition of clay fraction of successive layers of Tyddyn Llwydyn (Powys) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	46·6	33·3	12·6	2·38	1·92
II	45·5	33·2	13·4	2·33	1·85

The evidence for eluviation is small both from the field appearances and also from the analytical figures, but there is a small tendency for enrichment of sesquioxides in the subsoil. The slighter eluviation of the Tyddyn Llwydyn profile compared with the Cefn and Brithdir profiles may be connected with the better base status of the parent material.

Drift and hillwash soils. Three profiles from cultivated drift or hillwash soils are now presented. It should be noted that the dividing line between sedentary and drift soils is not hard and fast. Only the shallowest sedentary soils can be assumed free from admixture of drift.

Llanfair P.G., Anglesey. Altitude: 120 ft. Rainfall: 38 in. Parent material: schistose glacial drift. Gaerwen soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–9	Dark brown stony loam	2·9	5·40
II	9–15	Brown stony loam	2·05	5·64
III	15–36	Reddish brown stony loam	1·1	5·94
IV	36–48	Yellowish brown stony loam	n.d.	5·80

Table XIV. *Composition of clay fraction of successive layers of Llanfair P.G. (Gaerwen) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	43·9	31·2	16·1	2·39	1·80
II	45·1	31·4	16·7	2·47	1·85
III	44·8	31·6	16·6	2·41	1·80
IV	45·6	29·4	13·2	2·64	2·05

There is little difference in composition down to 36 in., but the material below this depth is definitely more siliceous in character, and may well represent *C* horizon material. If this be the case, the material down to 36 in. must be regarded as corresponding to *B* horizon material. An *A* horizon is not indicated, and the suggestion is that it has been removed by erosion in the past.

St Mary's College, Bangor. Rainfall: 40 in. Parent material: volcanic ash. Ebenezer soil series.

Horizon	Description	Organic carbon	*p*H
I	Dark brown loam	3·6	6·2
II	Brown loam	n.d.	5·8
III	Light brown stony loam	n.d.	6·2

Table XV. *Composition of clay fraction of successive layers of St Mary's College (Ebenezer) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	43·9	31·5	14·3	2·37	1·79
II	43·6	32·5	15·7	2·28	1·74
III	43·8	33·2	14·9	2·24	1·74

The clay fraction shows a reasonable constancy in composition with a suggestion of sesquioxide eluviation.

Aber, Caernarvonshire. Altitude: 90 ft. Rainfall: 40 in. Parent material: hillwash from Ordovician shale. Penrhyn soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–9	Brownish grey stony loam	2·4	5·4
II	9–18	Greyish brown stony loam	1·6	6·4
III	18–36	Light brown slightly mottled stony loam	0·9	7·4
IV	36–48	Similar to III	0·35	n.d.
V	> 48	Brown heavy stony loam	0·2	n.d.

Table XVI. *Composition of clay fraction of successive layers of Aber (Penrhyn) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	46·4	32·9	11·0	2·40	1·97
II	46·5	33·8	11·2	2·34	1·93
III	45·8	34·5	11·3	2·33	1·86
IV	48·2	33·5	8·7	2·44	2·10
V	46·5	32·3	11·0	2·44	2·01

The impression given is of a fairly constant composition for the clay fraction. The differences are not sufficiently definite to give any marked evidence of eluviation. The slightly higher figure for the silica-alumina and silica-sesquioxide ratios in the lower layers may be due to an admixture of a more siliceous Triassic Boulder Clay which occurs at about 5 ft., or it may represent *C* horizon material.

The three drift profiles resemble one another in the less marked differentiation of horizons, judged by the composition of the clay fraction, as compared with the sedentary profiles. It may be conjectured that this is partly due to their better base status, and possibly to the fact that their greater depth, by encouraging deep rooting, promotes a circulation of basic constituents, thus counteracting the degeneration which favours eluviation. With the shallower sedentary profiles bases are more readily washed down below the level from which they can again be brought to the surface by the action of plants or by capillary rise.

Profiles with impeded drainage.

Three profiles with impeded drainage are now presented. A large proportion of the area of North Wales must be assigned to this class. Whilst large extents of such soils are uncultivated, considerable areas, particularly of soils lying on slopes, have been artificially drained and are now under cultivation. Wet soils may be broadly subdivided into those lying in valley bottoms and hollows, and those lying on slopes. In the former case, the water table is never far below the surface, and flooding may occur in wet weather. In the latter case, the impeded drainage is due to the presence of relatively impervious layers. In dry weather, therefore, such soils may actually suffer from drought, and possibly more than the well-drained soils by reason of the restriction of root range. The bottom soils differ also from the slope soils in that they have received contributions from the soil of the uplands either by deposition from flood water or from rain wash.

Llanfair P.G., Anglesey. Altitude: 100 ft. Rainfall: 38 in. Parent material: drift from mica schist. Gesail soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–10	Greyish loam	3·6	6·9
II	10–20	Greyish mottled micaceous loam	0·8	8·0
III	20–30	Grey and brown micaceous heavy loam	1·4	8·0

Table XVII. *Composition of clay fraction of successive layers of Llanfair P.G. (Gesail) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	47·6	32·0	11·3	2·50	2·05
II	45·8	29·6	15·0	2·63	1·99
III	45·6	29·2	14·6	2·66	2·02

Although there is a greater content of ferric oxide in the lower layers, the silica-sesquioxide ratio does not markedly change with depth. The alkaline character of the lower layers should be noted. This may be attributed to calcareous ground water, for the parent material itself is non-calcareous.

Belmont, near Bangor. Altitude: 210 ft. Rainfall: 40 in. Parent material: boulder clay, mainly of volcanic ash material. Sion soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–10	Grey heavy loam	4·8	7·3
II	10–18	Grey mottled heavy loam	0·7	7·8
III	18–30	Grey mottled clay	0·8	8·0

Table XVIII. *Composition of clay fraction of successive layers of Belmont (Sion) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	51·7	30·15	8·4	2·92	2·48
II	49·9	30·25	10·6	2·81	2·30
III	47·9	27·1	12·6	3·01	2·31

This profile is more siliceous throughout than any of the profiles from the soils with free drainage. In spite of the brown mottling in the lower layers, there is no great degree of eluviation of the sesquioxides. The ferric oxide is, however, definitely higher in the lower layers, which correspond in appearance and origin with the "Glei" horizon of continental writers. As in the preceding profile, calcareous ground water has resulted in an alkaline reaction.

Tyddyn Llwydyn, Bangor. Altitude: 80 ft. Rainfall: 40 in. Parent material: Boulder clay from Ordovician flags. Cegin soil series.

Horizon	Depth (in.)	Description	Organic carbon	*p*H
I	0–8	Grey heavy loam	5·30	6·1
II	8–20	Grey mottled clay	2·47	5·8
III	>20	Grey very mottled heavy clay	1·06	5·7

Table XIX. *Composition of clay fraction of successive layers of Tyddyn Llwydyn (Cegin) profile.*

Horizon	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	SiO_2/Al_2O_3	SiO_2/R_2O_3
I	52·9	33·3	6·4	2·70	2·40
II	52·9	32·9	6·3	2·73	2·43
III	49·6	32·2	8·2	2·62	2·26

Comparatively little difference in composition is shown between the layers of this profile. There is a slight increase of ferric oxide in the lowest layer. The mottling in the second layer is not accompanied by any increase in the ferric oxide content.

The data for the last three profiles with impeded drainage are particularly instructive. In no case is there any decided difference between the composition of the clay fraction in successive layers, and this in spite of the fact that marked mottling is shown in the lower horizons. The differences which do occur indicate a slight eluviation of the sesquioxides. It is to be noted that the Belmont and Tyddyn Llwydyn profiles are from valley bottoms, whilst the Llanfair profile is from a wet boulder clay slope. Whilst in the last profile the silica-sesquioxide ratio approximates to 2·0, in the other profiles the clay is markedly siliceous. This and the general occurrence of grey soils in wet bottoms and hollows suggests the possibility that such soils include *A* material washed down from the ad-

jacent uplands. The mottling which is seen in the lower horizons of impeded drainage profiles is quite distinct from the marked accumulation of ferric oxide in the *B* horizon of upland soils for, on examination, it is seen that the brown colour is only superficial on the soil crumbs. This is reflected in the comparatively small accumulation of ferric oxide indicated by the analytical figures.

GENERAL DISCUSSION.

Viewing the soils of North Wales as a whole and restricting attention in the first instance to those with free drainage, one notices that the prevailing soil colour is brown or reddish brown, except in the case of poor heath soils which show a podsolised layer. The brown colour is often somewhat masked by the presence of organic matter, but becomes apparent after treatment with hydrogen peroxide. The average SiO_2/R_2O_3 ratio for twenty-eight cultivated soils derived from consolidated or crystalline rocks was found to be $1{\cdot}85 \pm 0{\cdot}12$, whilst the corresponding SiO_2/Al_2O_3 ratio was $2{\cdot}4 \pm 0{\cdot}16$. This is in good agreement with the results of other workers for soils derived from primary weathering, cited by Robinson and Holmes (10).

The question arises whether a complex, having a silica-sesquioxide ratio approximating to 1·85, represents the normal product of weathering under North Welsh conditions. This cannot be strictlyt he case since, in such profiles as have been examined, smaller silica-sesquioxide ratios are found in the subsurface horizons, indicating a certain degree of eluviation of sesquioxides. It may be doubted, indeed, if it is possible to discover the unchanged product of contemporary weathering, since the clay of deep boulder clay deposits may be the product of Tertiary weathering. Also it is not improbable that chemical weathering and eluviation proceed *pari passu*. Robinson and Holmes (10) have examined the possibility that the clay complex may be a mixture of two hydrous silicates, $Al_2O_3.2SiO_2.xH_2O$ and $Fe_2O_3.2SiO_2.xH_2O$. Whilst they conclude against this hypothesis, the present writer (5) has suggested that certain of the higher silica-sesquioxide ratios found by them occur in soils derived from alluvia and unconsolidated deposits, where a concomitant precipitation of silica from terrigenous waters may be assumed. Higher silica-sesquioxide ratios in arid soils may be due to incomplete removal of the silicic acid liberated in silicate hydrolysis. The work of Robinson and Holmes (10) and that of Marchand and Van der Merwe (11) supports the view that the clay complex contains a hydrous ferric silicate, and that iron in the clay is not there simply as adventitious ferric oxide.

Bearing these facts in mind, the results from North Wales are not inconsistent with the hypothesis that the primary weathering product is a mixture of hydrated silicates having the general formula $R_2O_3.2SiO_2.xH_2O$. Variations from the silica-sesquioxide ratio demanded by this hypothesis are to be expected owing to eluviation and erosion. Eluviation will tend to enrich the subsurface layers in sesquioxides, whilst erosion will tend to remove the superficial horizons which would otherwise show a silica sesquioxide ratio greater than 2·0.

That erosion has been an important factor in soil profile development in North Wales in past ages can scarcely be doubted. The clearing of deciduous forests which originally covered the country, and the institution of arable cultivation probably induced, under the high rainfall and with the steep topography, erosion similar to that which is evident in the Eastern United States at the present day. The subsequent establishment of grassland conditions and the building up of the organic content of the soil has now reduced erosion to almost negligible dimensions. The deep accumulations of hillwash and bottom soils remain as evidence, and it is noteworthy that the bottom soils are grey in colour and show a high silica-sesquioxide ratio, such as might be expected in *A* horizon material.

Reconstructing the history of our North Welsh soils, it may be conjectured that, under the conditions obtaining in the area, the primary product of weathering from crystalline or consolidated rocks is a mixture of hydrous silicates, having the general formula $R_2O_3.2SiO_2.xH_2O$. In primitive times, under a deciduous forest cover extending up to about 1700 feet, a podsolic type of profile was developed characterised by almost complete leaching out of the alkali and alkaline earth bases and a moderate degree of eluviation of the sesquioxides. During primitive and mediaeval times the forests were cleared, arable culture was instituted, and erosion removed the slightly bleached *A* horizon, most completely in the uplands where it was most strongly developed, less completely in the lowlands where the podsolisation was probably less intense. Pasture conditions were established on what was originally the *B* horizons in the uplands, and on the *B* horizon mixed, doubtless, with some *A* horizon in the lowlands. Under pasture conditions, the higher lands would gradually deteriorate in base status with a resultant development of secondary podsols under heath vegetation. The change from grass heath to heather bilberry heath can actually be demonstrated during the space of the last century in old cultivations. In the lowlands, the degeneration which results in podsolisation is held in check by cultivation and manuring. The *A* horizon material from the uplands removed by erosion was, to a large

extent, lost altogether by river transport to the sea, but accumulated to some extent in hollows now filled with grey soils under a rushy vegetation.

It remains to be noticed that wherever owing to the thinness of the soil or the prevalence of rock outcrops, lowland soils remain unimproved, the tendency to podsolisation can be observed, sometimes resulting in the development of a marked peaty layer overlying or masking a bleached horizon. In the writer's opinion both these and the upland podsols are really secondary in character. In the better type of grass heath intermediate between the cultivated lands and the true heath, the soils are generally of a brown or reddish brown colour, and probably represent profiles which have been truncated by removal of the *A* horizon material, but in which the secondary podsolisation has not yet become marked. In the absence of any ameliorative measures, these also may be expected to degenerate to heath and to develop podsol profiles.

In an earlier publication (12), the writer suggested that the soils of North Wales were generally immature. The views expressed in the present paper contradict the earlier hypothesis, and it is, therefore, necessary to state the grounds on which this change of opinion is based. One of the main reasons for the view that our soils were immature was the small proportion of clay found by the older method of mechanical analysis. This suggested that soil development had progressed little beyond the stage of mechanical disintegration. With the use of methods involving complete dispersion, however, it was found that Welsh soils were considerably richer in clay than had appeared formerly. This involved the abandonment of the view that the soils in question were skeletal. Assuming a moderate development of chemical weathering, the choice appeared to lie between the hypothesis that the clay fraction of North Wales soils represents the first product of weathering under local conditions, and the hypothesis put forward in the present paper which postulates modification by eluviation and erosion and which, with a fair economy of assumption, gives a reasonable account of the differences actually found.

The clay fraction as an aid to series definition.

The present writer (13) has suggested the adoption for soil survey purposes of a system of classification into soil series based on that in use in the United States Soil Survey. The criterion is the soil profile, and the series are grouped into suites consisting of soils derived from the same or similar parent material. It is instructive to compare the data for the composition of the clay fraction for different series in the same suite. The

Powys suite, derived from non-calcareous shales, flagstones, mudstones, etc., of Cambrian to Silurian age affords an example. In the Powys podsol series exemplified by the Cwm Du and Aber profiles an *A* horizon with a high silica-sesquioxide ratio is succeeded by a *B* horizon showing a much lower silica-sesquioxide ratio. In the Powys grass heath series as exemplified by the Dolwgan and Aber grass heath profiles the silica-sesquioxide ratio is low throughout the profile. In the Powys series (sedentary soils under cultivation) the silica-sesquioxide ratios both of soil and subsoil are not far below 2·0, and show a slight but definite decrease from soil to subsoil. The Penrhyn series shows a fairly constant silica-sesquioxide ratio through a depth of over 4 feet. Lastly the profile of the Cegin series shows a high silica-sesquioxide ratio for the clay fraction with comparatively little change with increasing depth. A similar picture is presented by the other suites. It is evident that, in the profiles discussed in the present paper, the affinities between soils formed from different parent materials under similar conditions are closer than those between soils derived under different conditions from the same parent material. An extension of this enquiry would doubtless reveal important distinctions due to parent material. Thus, in a Keuper Marl profile from Warwickshire furnished by Mr G. H. Gethin Jones, the silica-sesquioxide ratio remains fairly constant down to 36 in. at 2·72–2·78. Surface soils from the Triassic and later formations of South Wales showed ratios varying from 2·46 to 5·47, the latter figure relating to a Lower Lias soil. These high ratios are characteristic of soils formed from materials originally deposited from water, and in which the original colloidal material has persisted. They resemble, in fact, contemporary alluvial and estuarine deposits. The high proportion of silicic acid is considered by the writer to be due to concomitant precipitation of silicic acid which is derived ultimately from silicate hydrolysis. Thus, in the precipitation of sediments, the desilicification characteristic of silicate hydrolysis is in some degree repaired.

It seems clear from these considerations that a study of the silica-sesquioxide ratio of soil profiles will provide an important aid in the definition of soil series. In a wider aspect, the composition of the clay fraction may assist in the definition of the greater world groups. Martin and Doyne (14) have shown that lateritic soils can be defined on the basis of the composition of the clay fraction. The figures of W. O. Robinson and R. S. Holmes (10) suggest that arid soils are characterised by a high silica-sesquioxide ratio. This may be attributed to incomplete removal of silicic acid liberated in hydrolysis.

There remains one important aspect of the study of the clay fraction, and that is its bearing on soil properties. Joseph and Hancock (15) have shown that moisture-equivalent and other important physical properties can be correlated with the silica-alumina ratio of the clay fraction, the more siliceous clays being more highly colloidal in character. Similar results have been obtained by Anderson and Mattson (16), and by Bennett (17). We may thus distinguish two extreme types of clay, one the siliceous type with a high silica-sesquioxide ratio in which colloidal properties such as moisture retention, swelling, and base exchange capacity are strongly marked, and the other of the lateritic type in which the colloidal properties are much less strongly developed. It is reasonable to conjecture that these differences affect the reactions of soils with fertilisers, and also their relationships to plant growth.

Position of North Welsh soils in a world classification.

Sufficient evidence has been adduced in the present paper for the podsolic character of the soils of North Wales. Adopting the classification of Marbut (18) they belong to the pedalfers and, probably, to the subdivision of brown forest soils, also known as brown earths. These are soils in which there is a complete leaching of alkaline and alkaline earth bases from the soil profile and in which there is a slight eluviation of the sesquioxides. They are developed under deciduous forest. The data for temperature support this view, but the humidity, expressed by the $N - S$ factor, is rather greater than the upper limit assigned by Jenny (19) for the brown earths.

The assignment of the soils of North Wales to the brown earths must, however, be qualified by certain considerations which will now be discussed. In the first place, the influence of the parent material must be taken into account. Generally speaking, the soils in question are derived by primary weathering from lower Palaeozoic sedimentary, igneous, or metamorphic rocks or their associated drifts. They are predominantly loams and are, on account of their base reserves, more resistant to eluviation than soils of a more sandy character. It is scarcely to be doubted that if light sandy soils occurred in our area under conditions favourable for profile development, their character would be more definitely podsolic. In south-east England, where the climate is much less humid, typical podsols are found on the light Bagshot Sands. In defining the conditions under which a particular type of soil profile should occur, it is necessary, therefore, to consider the geology along with the climatic factors. In a

typical brown earth region, widely differing soil types may be encountered corresponding with differences in the local geology. In this connexion, it may be mentioned that there are in North Wales red soils associated with the Carboniferous Limestone, which may prove on examination to resemble the terra rossa of the Mediterranean region.

The present aspect of soils in North Wales has been affected by erosion. In the absence of any primitive forest, except in situations which preclude mature profile development, the character of the original soil profile can only be the subject of conjecture. In the opinion of the writer, all existing profiles are more or less truncated. This truncation is most marked in the upland regions, where the soils up to the lower limit of the heaths have a well-marked *B* horizon character. The podsol profiles of the heaths are probably of secondary origin and mark the deterioration of grass heath to heath.

It should be noted that North Wales at the present day is predominantly an area of grassland, and that the soils have to this extent affinities with the prairie soils, containing as they do large proportions of organic matter. The grassland condition is, however, artificial, and in the absence of grazing by animals, scrub, and, ultimately, forest would again establish itself.

It remains to discuss the affinities of the Welsh brown earths to adjacent soil groups. The continental brown earths border on the black earths on the side of decreasing humidity and on the red earths with increasing temperature. In North Wales both humidity and temperature are higher than in the typical mid-European brown earth region. In the range of temperature associated with this soil group, it is probable that our soils are near the upper limit for both factors. The transition to podsols can be observed in the wet uplands. It may be conjectured that, if soil formation could be followed on the same parent material to a warmer climate with the same humidity, the transition would be to red earths. Red soils occur on the Carboniferous Limestone of North Wales, and further studies may establish their affinity with the red earths of the Mediterranean region. The geological factor cannot be eliminated, and fuller investigation may prove the co-existence under identical climatic conditions of podsols, brown earths and red earths.

The writer is indebted to his colleagues in the soil survey of Wales, Messrs J. O. Jones (now of Long Ashton), D. O. Hughes, G. H. Gethin Jones (now of Wye), Brynmor Jones (now of Nigeria), and W. G. D. Walters for the collection of most of the profiles discussed in this paper, and to Dr R. Alun Roberts for data on natural vegetation.

SUMMARY.

1. The changes in composition of the clay fraction in different horizons of some typical North Welsh profiles have been studied.

2. The changes in the molecular ratio of silica to sesquioxides ($SiO_2/Al_2O_3 + Fe_2O_3$) throughout a soil profile afford an indication of the nature of the eluviation which has affected the mineral portion of the soil.

3. The data for North Welsh soils indicate a general tendency to enrichment of the lower layers in sesquioxides, particularly ferric oxide, at the expense of the surface layers.

4. The silica-sesquioxide ratio of the clay fraction is an important aid to series definition.

5. The soils of North Wales, particularly in the uplands, have probably been subjected to considerable erosion in the past. The profiles are therefore considered to be more or less truncated.

6. Under North Welsh conditions, the soil profile appears to be of the brown earth type, with podsol development in the uplands under heath vegetation.

7. The importance of the parent material is emphasised. North Welsh soils are generally of a loam character. The absence of light sandy soils in situations permitting profile development precludes the occurrence of typical podsols in the lowlands.

8. The high content of organic matter, resulting from artificial grassland conditions, results in affinities with prairie soils.

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(*Received September 1st*, 1930.)

CAMBRIDGE: PRINTED BY
W. LEWIS, M.A.
AT THE UNIVERSITY PRESS